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ABSTRACTS

Babson, A. L., Read, P. A., and Phillips, G. E.: The importance of the substrate in assays of acid phosphatase in serum. Am. J. of Clin. Path. 32:1 83-87, July 1959.

A comparison is made between six generally used substrates for their relative specificity to prostatic and erythrocytic acid phosphatase. Results showed that a new substrate, alpha-naphthyl phosphate, was twice as specific as beta-glycerophosphate and 40-100 times as specific as all other substrates investigated. Acid phosphatase assays in serum should be specific for that enzyme arising from cancerous prostatic tissue. The substrate alpha-naphthyl phosphate* is specific for this enzyme.

Babson, A. L., and Read, P. A.: A new assay for prostatic acid phosphatase in serum. Am. J. Clin. Path. 32:1, 88-91, July 1959.

A new and specific method for the determination of prostatic acid phosphatase in serum is presented. The method is based on the use of a new substrate alpha-naphthyl phosphate which measures only prostatic acid phosphatase. The method is simple and rapid to perform with a minimum of manipulation. In this procedure the color developed represents prostatic enzyme activity alone. The reagents for this new method are available in convenient and stable form.*

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A. M. A.
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Official Organ of the AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

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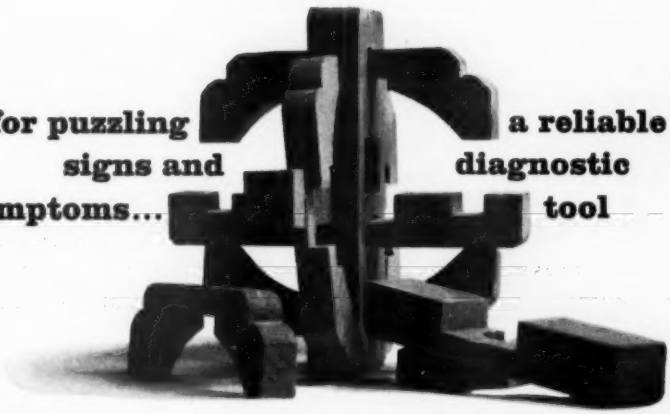

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PATHOLOGY

Electron Microscopy Studies of Butter and Corn Oil in Jejunal Mucosa

WILBUR A. THOMAS, M.D., and ROBERT M. O'NEAL, M.D., St. Louis

Recently we devised a dietary method for the production of coronary and renal arterial thromboses and myocardial and renal infarcts in rats,[1] hence providing for the first time a suitable experimental model for study of some of the fundamental processes involved in arterial thrombosis. The infarct-producing diets we use usually include a high percentage of saturated fats plus cholesterol, bile salts, and thiouracil. Light microscopy studies have shown that large quantities of the fat are deposited in all organs. If corn oil, a highly unsaturated fat, is substituted for saturated fats in the diet, the incidence of infarcts appears to be drastically reduced. In tissue sections stained for fat with oil red O we have been unable to detect any difference between the appearance of fat in rats fed butter, a highly saturated fat, and rats fed corn oil, a highly unsaturated fat.[2]

We decided that electron microscopy studies of osmium-fixed tissues might provide further information and proceeded to examine sections from various organs of rats that had been given infarct-producing diets containing various fats. Osmophilic materials, presumably lipids and lipoproteins, were readily demonstrable in the blood and in various tissues. The osmophilic material assumed many shapes and densities, and some tended to be repeated often enough to suggest that they represented similar compounds.[3] Because of the complexity of the diets, it was impossible to identify specific substances. Therefore, we decided that examination of a simpler system was necessary. As an initial step, normal fasting animals were fed single meals of butter, corn oil, sugar solution, or water by gastric tube, and then sections of jejunal mucosa were examined by phase and electron microscopy for the presence of absorbed substances.

The purpose of this report is to describe and illustrate the osmophilic substances observed in the jejunal mucosa of these animals.

Materials and Methods

Healthy male Wistar albino rats, weighing 100-200 gm., that had been maintained on Purina pellets, were given only water for 24 hours prior to the experiment. Each rat was fed 10 ml. of corn oil (Mazola), melted commercial butter, syrup (Karo), or water (Table) by transoral gastric intubation. The rats were killed with ether inhalation or by a blow on the head at either 10 to 20 minutes or one hour after intubation; the abdomen was opened, and sections of jejunum were taken for electron and light microscopy. Tissues for electron microscopy were placed in Dalton's osmium tetroxide fixative for one to two hours, with frequent changes of the osmium, dehydrated in

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From the Department of Pathology, Washington University School of Medicine.

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graded concentrations of ethanol, and embedded in methacrylate (seven parts butylmethacrylate to one part methylmethacrylate). Thin sections were cut on a Porter-Blum microtome and examined in an RCA Model EMU-3B electron microscope. Thicker sections were examined by phase microscopy. Carbowax (polyethylene glycol)-embedded sections of cobalt-formalin-fixed tissues were stained with oil red O for light microscopy.

Results

The results are summarized in the Table and illustrated in the electron micrographs (Figs. 1-8). Osmophilic material suggestive of lipids was not observed in the mucosal cells of the fasted rats fed syrup or water.

The ultrastructure of intestinal lining cells has been described previously,[4] and only pertinent points will be reviewed here.

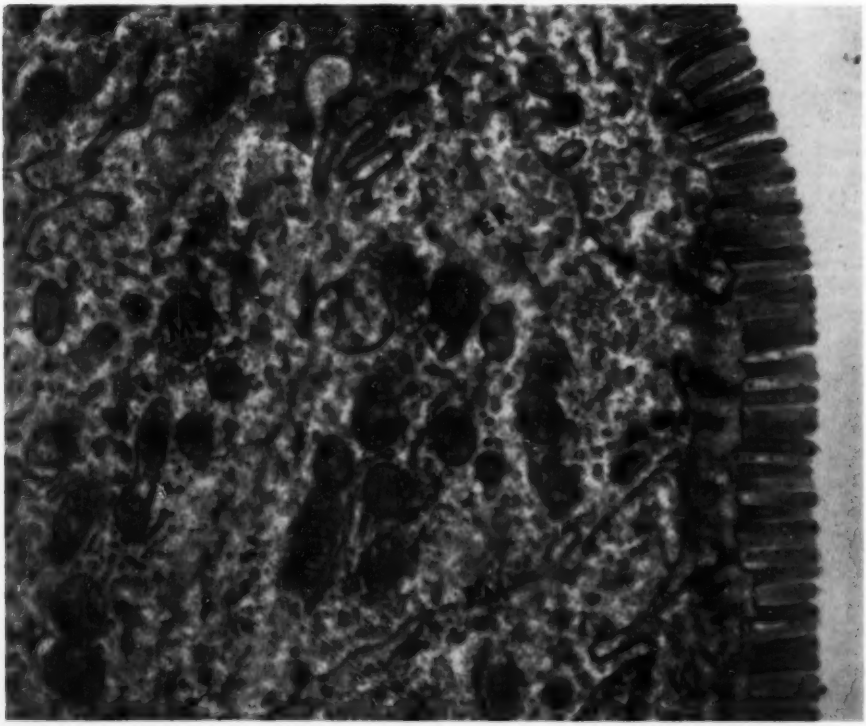
Results of Feeding Various Meals

Type of Meal	Time, Min.*	No. of Rats	No. with "Lipid" †
Corn oil	10-20	4	3
Corn oil	60	6	6
Butter	10-20	5	1
Butter	60	6	6
Sugar	60	2	0
Water	60	4	0
Fasted only	--	1	0

* Interval between feeding and death.
† Number with osmophilic masses in mucosal epithelium.

The luminal surface of absorptive cells of the jejunal mucosa is composed of closely spaced microvilli about 1,400 A. in diameter (Fig. 1). The cytoplasm within the microvilli and immediately beneath them is devoid of mitochondria, but there is a dense, rather

Fig. 1.—Jejunum of a normal rat fasted for 24 hours prior to death. Portions of at least four mucosal epithelial cells are seen, separated by their tortuous plasma membranes (*PM*). Mitochondria (*M*) are frequent and contain rather closely packed cristae. The small vesicular structures scattered through the cytoplasm are cross sections of the endoplasmic reticulum (*ER*). The microvilli forming the surface of the intestinal lumen are closely spaced. Beneath the microvilli is an osmophilic band, representing the "terminal web." × 20,000.



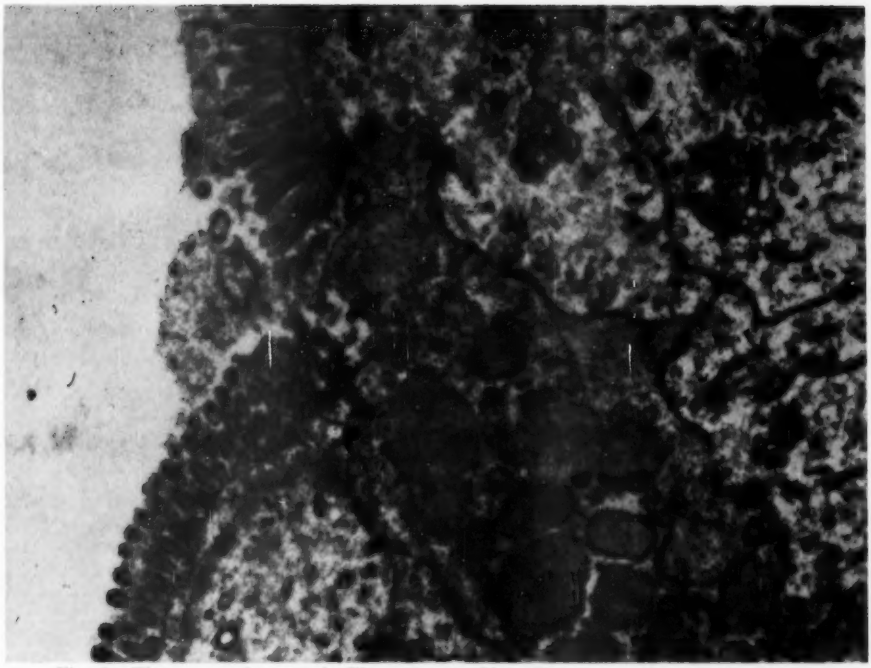
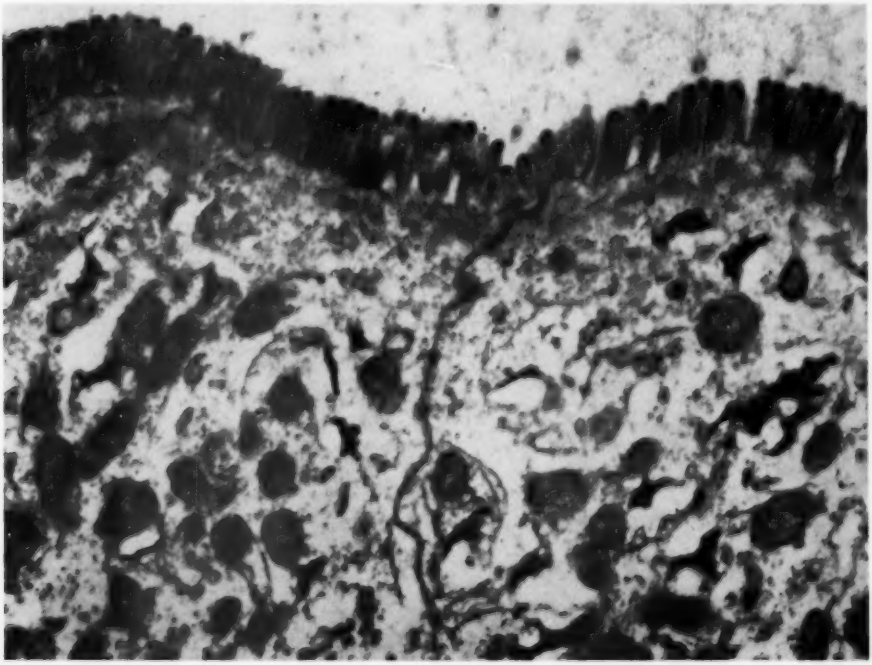


Fig. 2.—Tissue near that seen in Figure 1. A goblet cell appears to be emptying its content of mucogen droplets into the intestinal lumen. × 20,000.

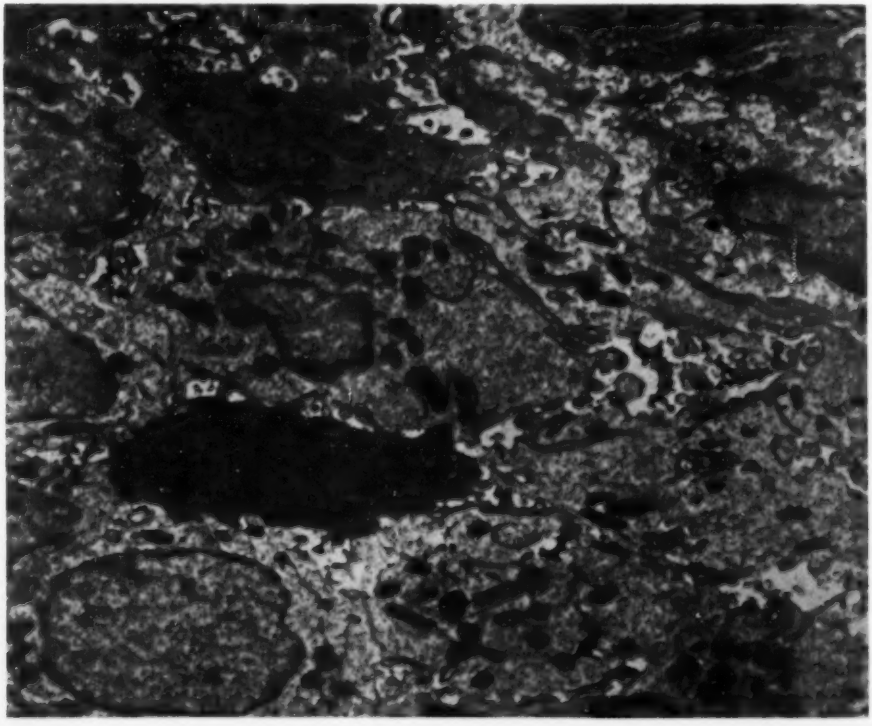
Fig. 3.—Superficial portions of jejunal mucosal cells of a rat that was fed butter one hour before death. The lipid is seen as black (deeply osmophilic) bodies with bizarre shapes about the size of the mitochondria, and is usually adjacent to a clear space, which is not seen in fasted animals. The appearance of the fat may be partially due to artifact, resulting from tissue fixation, but is apparently dependent on the nature of the fat. × 20,000.



well-defined osmophilic band in the cytoplasm subjacent to the microvilli. This band corresponds to the "terminal web" described by Saur [5] in a study with the light microscope. Beneath the terminal web, endoplasmic reticulum and mitochondria are dispersed through the upper apical cytoplasm of the cells. The membranes comprising the Golgi apparatus, as well as the nucleus of the cell, lie deep to the area illustrated in Figure 1.

Osmophilic masses were observed in the epithelial cells of the jejunal mucosa in all rats fed corn oil and killed in 10 to 20 minutes and in one of five rats fed butter and killed in 10 to 20 minutes. In rats fed corn oil, the osmophilic material (lipid) was observed in the lumen and within the mucosa. In both places the lipid took the form of rounded, jet-black masses, varying in size from 300 A. to 2μ. Much of the lipid in the cytoplasm of the mucosal lining cells was concentrated in a broad zone of the apical cytoplasm, which begins about 0.5μ below the microvilli; individual small droplets were surrounded by a membrane, which usually was rough-surfaced. Below the microvilli, and above the ill-defined zone containing lipid, there was always the well-defined layer of cytoplasm (containing the terminal web), which was free of lipid except for an occasional small droplet. Moderate quantities of lipid were found deeper in the cells and between mucosal cells. There were some lipid droplets among the microvilli. These droplets were invariably small, measuring 300 to 1,000 A. Apparently almost all the droplets were penetrated well by the osmium, because dropping out of centers was seldom ob-

Fig. 4.—A deeper portion of jejunal mucosa from the same rat as that in Figure 3; the plane of section is parallel to the surface of the epithelial cells. Spaces exist between the plasma membranes of adjacent cells, and these intercellular "canals" contain aggregates of small lipid bodies. × 8,600.



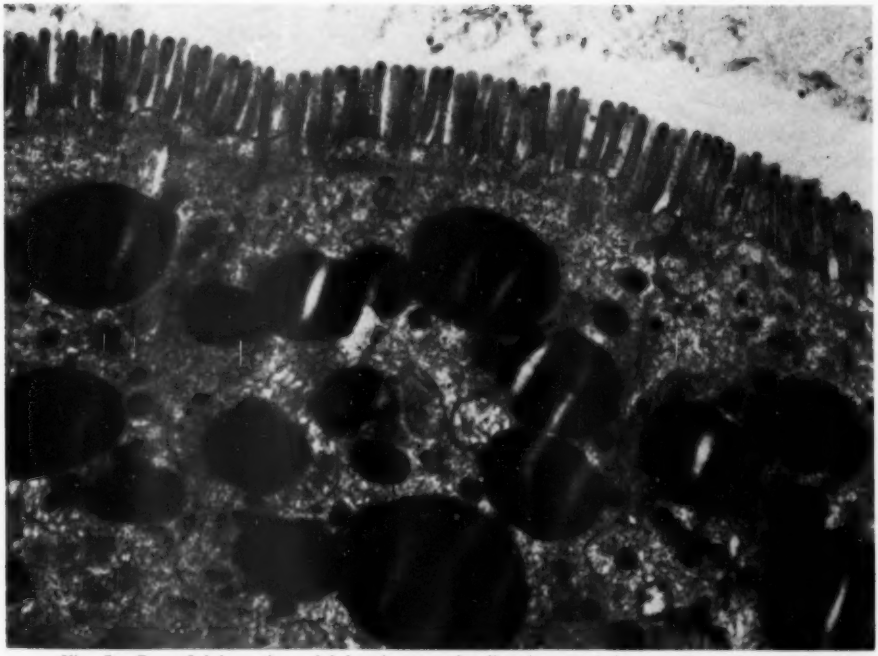
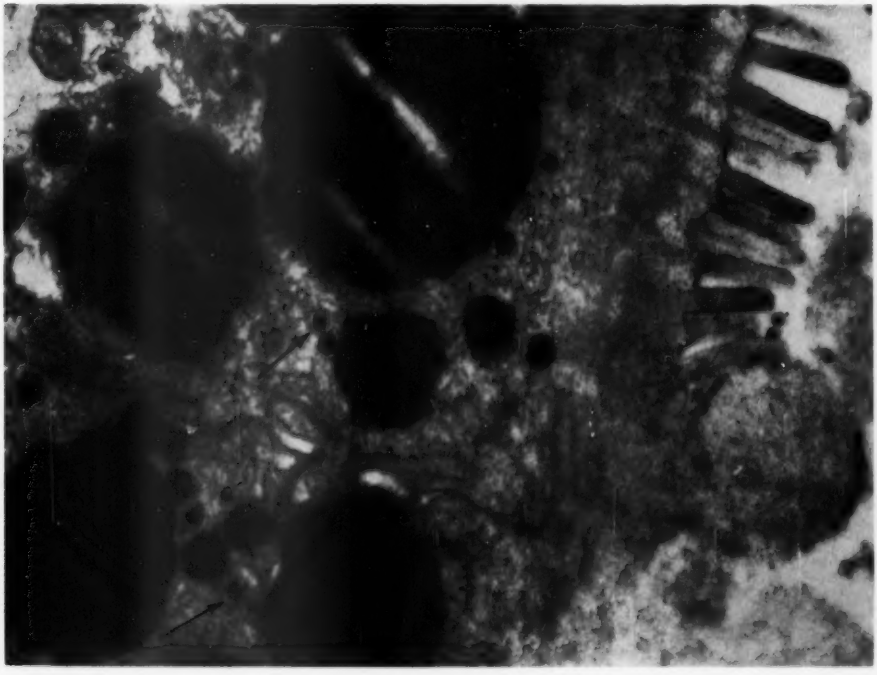


Fig. 5.—Superficial portion of jejunal mucosal cells of a rat fed corn oil one hour prior to death. The large, spherical lipid bodies appear homogeneous except for compression lines, and are spectacularly different from those seen in the rats fed butter. × 20,000.

Fig. 6.—A higher magnification of mucosa from the same rat as that seen in Figure 5. Arrows indicate smaller lipid bodies, which appear to be surrounded by a membrane, probably that of the endoplasmic reticulum. The plasma membranes of the two adjacent cells form a smooth-surfaced membrane of double contour traversing the tissue from left center to right lower. × 36,000.



served and there were no holes elsewhere suggesting loss.

In rats fed butter, lipid was observed in the lumen and was present in the mucosa in much the same position as described above for corn oil. No round forms were seen; instead, irregular linear and crescent forms were usually found, often with adjacent large clear areas, suggesting that during the preparation of the tissues part of the lipid had dropped out or become distorted. When larger masses were present, they tended to have "feathery," irregular margins. The total quantity of lipid present appeared to be as great as in the rats fed corn oil.

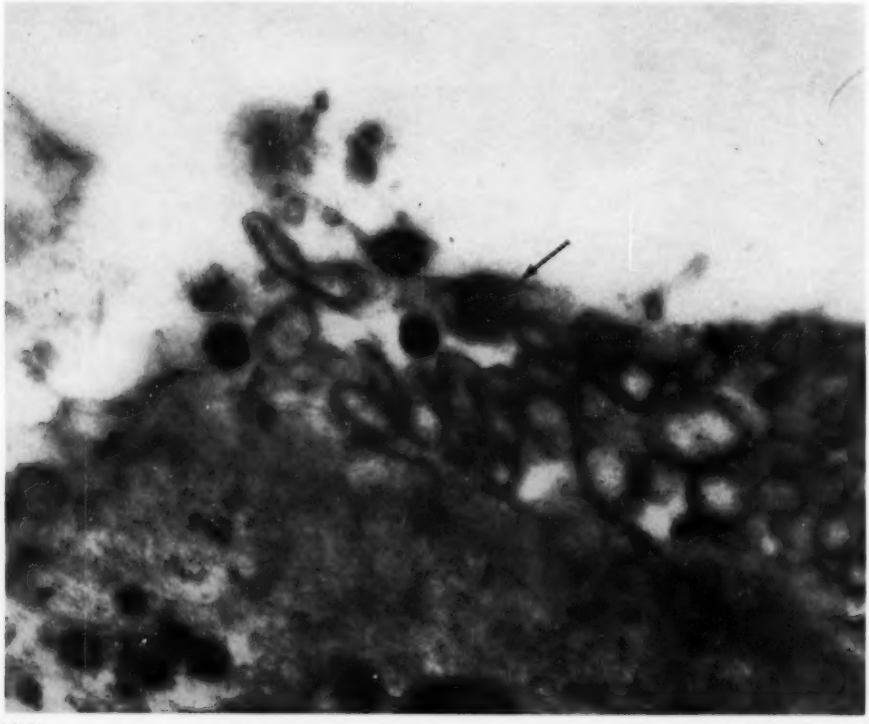
Under phase microscopy examination the appearance of "thick" (approximately 1μ) sections cut with the Porter-Blum microtome corresponded to that observed with the electron microscope. Osmophilic masses were observed only in those rats in which similar masses were seen by electron microscopy. The morphologic features were naturally not as distinct with phase microscopy, but we could observe rounded intracellular masses in the rats fed corn oil and irregularly contoured masses with surrounding clear spaces in the rats fed butter.

Examination of sections cut for light microscopy (4μ) and stained with oil red O revealed the presence of fat in the appropriate sections. However, distinguishing characteristics between the rats fed butter and the rats fed corn oil were not discerned with the oil red O stain.

Comment

We have shown that lipids from two different sources appear different in the

Fig. 7.—Tangential section of mucosa of rat fed corn oil one hour before death. Fat droplets are present between the microvilli at the surface of this cell, and one (arrow) appears to lie within a microvillus. Note the hazy osmophilic band (terminal web) just below the base of microvilli; fat droplets are seldom seen in this zone, suggesting that transport through it is very rapid. × 36,000.



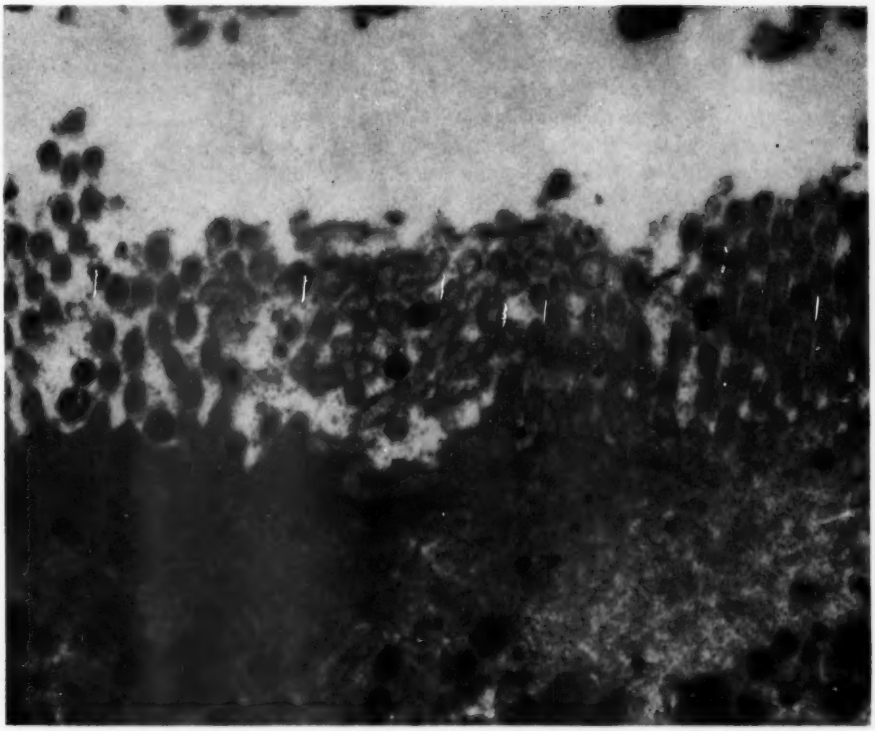


Fig. 8.—Same rat as that in Figure 7, showing fat particles between microvilli. This was the commonest position for fat that appeared to be entering the cell. Again note the relative sparsity of fat in the cytoplasmic zone immediately below the microvilli. × 36,000.

jejunal mucosa when examined with the electron microscope. One can easily differentiate rats fed butter from those fed corn oil by the appearance of the fat in the cytoplasm of the epithelial cells.

The uniformity of appearance of lipid in the jejunal mucosa with either corn oil or butter, although they differ greatly from each other, is of interest, since both these fatty substances are composed of several different fatty acids. Butter even contains a small amount of protein. Perhaps the electron microscopic appearance is a factor of some characteristic of fats, such as degree of saturation. Certainly degree of saturation is a likely factor, since osmium tetroxide combines readily with lipids containing double bonds and poorly, if at all, with saturated fats. Since butter contains many saturated fatty acids, the holes observed in butter-fed rats probably represent unfixed saturated fats that dropped out in the preparation of the tissue.

In another study now in progress, we are examining the jejunal mucosa of animals fed cottonseed oil, lard, hydrogenated cottonseed oil (Crisco), or coconut oil. In sections thus far examined, cottonseed oil is identical in appearance with corn oil. Lard, Crisco, and coconut oil appear similar in many respects to butter. More study will be necessary before we can determine with certainty whether each of these fatty substances has individual identifying characteristics. However, the ones we have examined thus far are consistent with the concept that the electron microscopic appearance in osmium-fixed sections is largely a factor of degree of saturation.

Even though the differing appearances of fats with differing degrees of saturation are due entirely to "artifactitious" changes

following osmium fixation, the differences are no less important or less reliable. Deane has suggested that the shape assumed by lipid droplets in response to fixatives probably reflects the nature of the lipids.[6]

Palay has suggested that the lipid droplets found among the microvilli (as demonstrated in Figures 7 and 8) represent the form in which fat is absorbed.[7,8] If this assumption is true, it tends to support the theory that fat is absorbed in particulate form from the lumen of the intestine; however, the maximum size observed thus far is not as great as that suggested by Frazer,[9] nor have "pores" been observed. Many of the droplets of lipid found in cytoplasm of the jejunal epithelium of rats fed corn oil are much larger than the droplets found between the microvilli, suggesting that small droplets coalesce within the cell to form, by comparison, huge droplets. The small fat droplets present in the cytoplasm are within membranous sacs, as described by Palay.[7] Although no definite, continuous membranes could be identified around the very large droplets in our preparations (Fig. 6), it seems likely that they lie within membranous structures.

The presence of lipid in the intercellular spaces (Fig. 4) indicates that part of the transport route is between the plasma membranes of adjacent mucosal cells. In general the fat in intercellular spaces was not near the mucosal surface, suggesting that the fat had not been absorbed directly into the space, but had entered the cell at the surface and then passed out again. This agrees with the interpretation of results of a somewhat similar study by Weiss.[4] However, the vacuoles he described in the upper apical portions of intestinal cells would appear, on the basis of our results, to be large lipid droplets artifactually distorted and only partially retained, rather than simply dilated Golgi vacuoles. That he fed cream to his experimental animals and used osmic-acid fixation of tissues supports this view. We have been unable to confirm his finding of 40 A. lipid droplets dispersed through the apical cytoplasm of absorptive cells; the droplets in our experiment appeared much larger, and many were of the size he described as dilated Golgi sacs.

The underlying purpose of our study was to gain a better understanding of the abnormal lipid deposits in rats on infarct-producing diets.[2,3] It is apparent that further studies must be done using many different fats, tracing the fats from jejunal mucosa to abnormal deposits, in order to achieve our goal. However, it is encouraging that morphologic differences between butter and corn oil are so readily demonstrable, even in the early stages of fat absorption.

Summary

We have recently devised a dietary method for the production of arterial thrombi and infarcts in rats. The infarct-producing diet most commonly used by us includes, among other ingredients, a high percentage of butter. Substitution of corn oil for butter has not produced infarcts, although abnormal deposits of fat in the tissues have appeared much the same with either source of dietary fat. In this study we have utilized the electron microscope to examine absorbed butter and corn oil in the jejunal mucosa of rats that were fasted for 24 hours prior to feeding of the fat. The electron microscopic appearance of absorbed butter in the jejunal mucosa was quite different than that of corn oil.

It seems likely that the difference in appearance of butter and corn oil in osmium-fixed tissue is due to the different degrees of saturation of the two fats. However, further studies must be done to establish this point.

Department of Pathology, Washington University School of Medicine.

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A Connective Tissue Disease with Pulmonary Fibrosis in Guinea Pigs

PAUL GROSS, M.D.; MARIAN L. WESTRICK, Ph.D., and JAMES M. McNERNEY, M.P.H., Pittsburgh

Under the title, "Pulmonary Fibrosis and Giant-Cell Reaction with Altered Elastic Tissue: Endogenous 'Pneumoconiosis,'" Walford and Kaplan [1] described a pulmonary interstitial fibrosis that had some resemblance to the pulmonary changes encountered in idiopathic pulmonary hemosiderosis and in some cases of severe mitral stenosis. Eleven cases of this peculiar fibrosis were discovered in a series of 7,000 autopsies reviewed, and a twelfth case had been supplied from another institution.

The etiology of this pulmonary disease was considered unknown, although various possibilities were discussed. Walford and Kaplan [1] concluded that the pulmonary change "is basically related either to an idiopathic structural defect in elastic tissue or to acquired damage of elastic tissue by immunologic metabolic, or perhaps inflammatory ('rheumatic pneumonitis') means."

During the course of investigations dealing with the pulmonary changes incident to the inhalation or injection of various dusts, a number of animals were encountered the lungs of which contained lesions strikingly similar to those described by Walford and Kaplan.[1] Since no such lesions could be found in a series of over 100 animals not exposed to these dusts, the probability exists that there was a cause-and-effect relationship between the experimental conditions and the lesions noted. If this relationship can be proved, further study of the lesions in animals may explain the lesions in human lungs. These lesions, in experimental animals, aside from their academic interest, therefore, may have clinical implications.

Submitted for publication March 20, 1959.

From Industrial Hygiene Foundation.

This work was partially supported by grants from Grinding Wheel Institute and Abrasive Grain Association, Owens-Corning Fiberglas Corp., and the National Institutes of Health (E1710).

Material and Methods

The animals which are the subject of this report belonged to two different dust-inhalation experiments, separated by a space of two years, and an intratracheal dust-injection experiment. The first of these experiments included 148 guinea pigs, divided into four groups of 32 to 36 animals exposed for 12 months in inhalation chambers to one of four dusts: namely, quartz, kaolin, aluminum oxide, and silicon carbide. Fifteen animals in each group received intratracheal injections of a weakly virulent strain of tubercle bacilli. After completion of the 12-month dust exposure, the guinea pigs were pastured for 12 more months, after which the survivors were killed. There was an additional group of 15 animals that had not been exposed to dust but had received intratracheal injections of the same suspension of tubercle bacilli.

In the second investigation, using 58 guinea pigs, three groups of 25, 17, and 16 animals, respectively, were exposed in a similar manner for 12 months to quartz, kaolin, and glass dust, respectively.

The third investigation involved intratracheal injections of 50 to 150 mg. of quartz dust that had been collected by electrostatic precipitation from the previously mentioned inhalation experiments and subsequently stored. In some of the guinea pigs examined from this series, the quartz dust had been injected in association with 0.005 to 0.03 mg. purified protein derivative U.S.P. (P.P.D.); in others, the quartz dust had been injected together with formalin-killed tubercle bacilli.

All guinea pigs used in the various investigations were males and were 6 to 8 weeks old when first exposed to dust or injected with it. Since all surviving animals were killed two years after the first exposure or injection, no animal was older than 26 months, and some were younger.

The average particle size of the dusts was less than 0.5μ, and the average dust concentration in the chambers varied from 25 to 50 mg/cu. m.

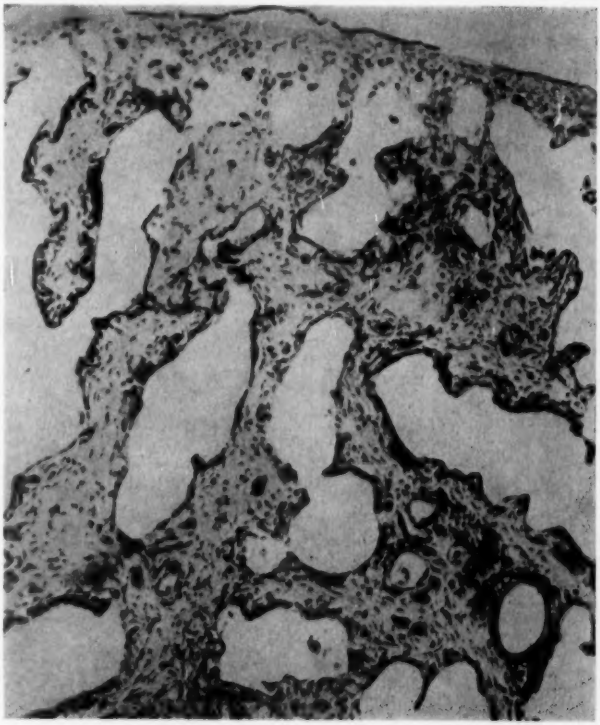

Fig. 1.—From guinea pig No. 335, exposed to kaolin dust for 12 months and then pastured for 7 more months. There is interstitial fibrosis, characterized by fibrillar stroma and plump fibrocytes. The outlines of bronchioles and alveolar ducts exhibit shallow or token indentations, which represent the sites of obliterated or partially obliterated alveoli. In some alveolar ducts even the token indentations have disappeared. The air spaces are incompletely delimited by a basophilic membrane. Hematoxylin and eosin; × 175.

For control purposes, the lungs and other tissues from 122 male guinea pigs not exposed to inorganic dust were examined. The ages of the control animals corresponded well to those of the experimental groups. None of them was younger than 8 months or older than 26 months.

The tissues were fixed in formalin or Bouin's fixative. In addition to hematoxylin and eosin, replicate sections were processed with the following: Weigert's elastic tissue stain with superimposed Van Gieson's stain, and counterstained with acidic potassium ferrocyanide, the resorcin-crystal violet method [3] for elastic fibers, the Gordon and Sweet method for reticulin fibers, and Von Kóssa's method for the demonstration of calcium. Some of the sections were incinerated and the topographic distribution of the acid-insoluble ash [3] was studied.

The silicon dioxide dust was subjected to x-ray diffraction and quantitative chemical analysis for iron. The kaolin and glass dusts were given qualitative tests for iron with acidic potassium ferrocyanide.

Results

General Findings.—Although the x-ray diffraction pattern did not indicate the presence of iron in crystalline form, chemical analysis proved that 8.8%±0.2% of the dust was iron. Both the kaolin and the glass dusts gave positive tests for iron.

Sections of lung stained by the acidic potassium ferrocyanide method demonstrated abundant iron associated with the various dusts. With the lapse of time during the holding period, the amount of demonstrable iron diminished.

The dust inhaled by, or injected into, the guinea pigs produced characteristic histologic responses.[4,5] The foci of connective tissue disease in the lungs were, in some instances, superimposed upon regions of dust deposition, but oftener the foci of connective tissue disease were independent of the dust deposits.

Fourteen guinea pigs were found which had prominent disease of elastic, and other, connective tissues. In every one of these animals the lungs were involved. Lesions of the trachea, aorta, myocardium, heart

Distribution of Lesions in Guinea Pigs with Connective Tissue Disease

Case No.	Guinea Pig No.	Age, in Mo., Killed or Died	Dust	Lung	Trachea	Heart		Aorta
						Aschoff Type	Calcification	
1	173	K 26	Quartz	+	+	+	—	+
2	253	K 14	Kaolin	+	+	—	+	+
3	303	K 26	Glass	+	—	+	—	+
4	306	K 21	Glass	+	+	+	—	+
5	307	K 21	Glass	+	+	+	—	+
6	322	K 25	Quartz	+	+	+	+	+
7	333	K 18	Kaolin	+	—	+	—	+
8	335	K 21	Kaolin	+	+	+	—	+
9	338	D 10	Kaolin	+	+	+	+	+
10	341	K 26	Kaolin	+	—	—	—	+
11	349	D 23	Quartz	+	—	—	—	—
12	350	K 24	Glass	+	—	+	—	—
13	390	K 15	Quartz	+	—	+	+	+
14	413	D 12	Quartz	+	—	—	+	+

valves, pulmonary artery, and other large arteries were also noted, but not in all of the 14 animals. These 14 guinea pigs represent an incidence of approximately 5% of 281 animals exposed to one of five different dusts in inhalation chambers or given injections of quartz dust intratracheally. Of the guinea pigs, 5 were from a total of 53 exposed to kaolin dust; 4 from a group of 16 exposed to glass dust, and 5 from a total of 135 animals exposed to quartz dust. Two of the last-mentioned number stemmed from a group of guinea pigs given intratracheal injections of 50 mg. of quartz dust plus 0.005 mg. P. P. D. The significant data are listed in the accompanying Table.

Grossly, the only demonstrable lesions were pigmentation of some of the lungs, due to dust deposition, and cylindrical aneurysmal dilatation of the aorta. Irregular granularity and pitting of the renal surfaces were noted in both the experimental and the control animals.

Microscopic Findings.—Trachea: The earliest lesions of the trachea involved the tunica propria, which became thickened and of hyaline character. In more advanced lesions, basophilia of the tunica developed, which was associated with the impregnation of calcium salts and occasionally also with iron; but in most instances stains for iron were negative in the trachea. These changes were accompanied by the deposition of a brittle, crystalline material that, at first, was colorless and translucent but later became impregnated with calcium and opacified. Deposits of this material were about 30μ thick and tended to form irregular, band-like masses, which were often associated with multinucleated giant cells. Rarely, small foci of calcification were found within the smooth muscle of the trachea.

Lungs: The most striking involvement consisted of great widening of alveolar walls by a relatively loose connective tissue that contained thick collagen fibers and irregular, thick, basophilic fragments of elastic fibers. The latter were often associated with multinucleated giant cells of foreign-body type. The giant cells contained no recognizable inclusions. Other components of the widened alveolar walls included plump, fibrocyte-type cells, scattered spheroidal bodies, 25μ to 40μ in diameter, and rare larger, irregular calcific foci. The spheroidal bodies were usually calcified, occasionally laminated, rarely colorless and translucent, and rarely impregnated with iron. It was common to find small alveolar remnants within the broadened interstitium. In some instances, these alveolar remnants were lined by metaplastic columnar epithelium, and in others a basophilic membrane, resembling an altered calcified elastic fiber, lined the diminutive air space. It was not unusual for the widened alveolar or atrial walls to exhibit

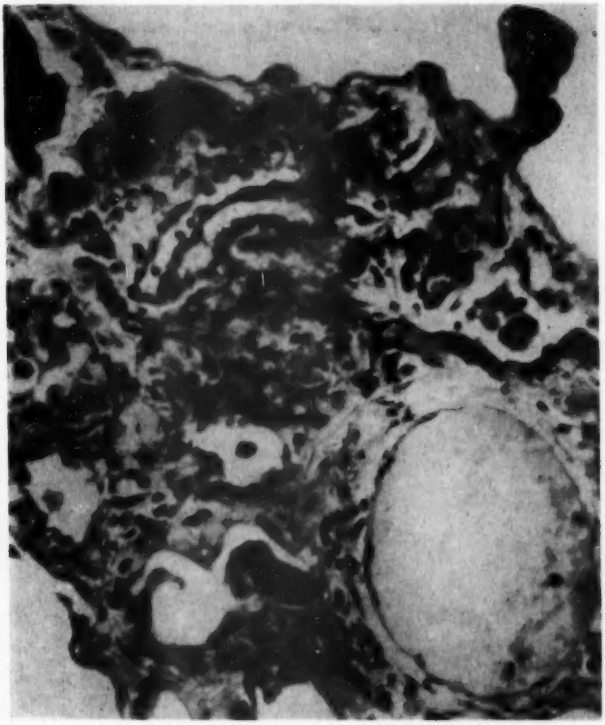

Fig. 2.—From same animal as in Figure 1. In addition to the fibrillar stroma and plump fibrocytes, the thickened walls contain irregular, coarse, wavy, curved, or angulated, deeply basophilic elastic fibers impregnated with calcium salts. Larger, irregular calcific foci are also present. The air spaces contain an occasional giant cell. Hematoxylin and eosin; × 440.

focal delimitation by similar prominent, basophilic membranes. The Von Kóssa stain indicated that these basophilic membranes and the thick, basophilic fragments of elastic fibers were impregnated with calcium salts. Iron was rarely demonstrable within these structures.

In order to learn more of the genesis of the lesions, attention was focused on those areas where there was transition from the abnormal to more normal alveolar walls. In some areas, the earliest change seemed to be a proliferation of alveolar cells with associated reticulin framework, growing into the alveolar lumen, and finally obliterating the latter completely, or nearly so. The elastic tissue degeneration, collagen formation, giant cells, spheroidal bodies, and calcium deposits apparently came later.

The lesions began in the walls of bronchioles and extended distally. It was, therefore, not surprising to find that the interstitial involvement followed the distribution of the bronchial passages. The thickening of the bronchiolar walls also came about by a proliferation of cells belonging to the alveolar walls contiguous with the bronchiolar wall. The cellular proliferation was accompanied by the growth of a reticulin framework. In some lobes of several animals, the interstitial fibrosis was so diffuse that no special distribution was discernible. Mostly, however, the involvement was focal, and in several animals the lesions were minimal.

The larger bronchi were mostly normal, but a few showed focal changes in the tunica propria, similar to those found in the trachea.

The pulmonary vessels within the lung were normal.

Arteries: The aorta was found involved in all but 2 of the 14 guinea pigs that demonstrated pulmonary lesions. The elastic lamellae were calcified. They had lost their

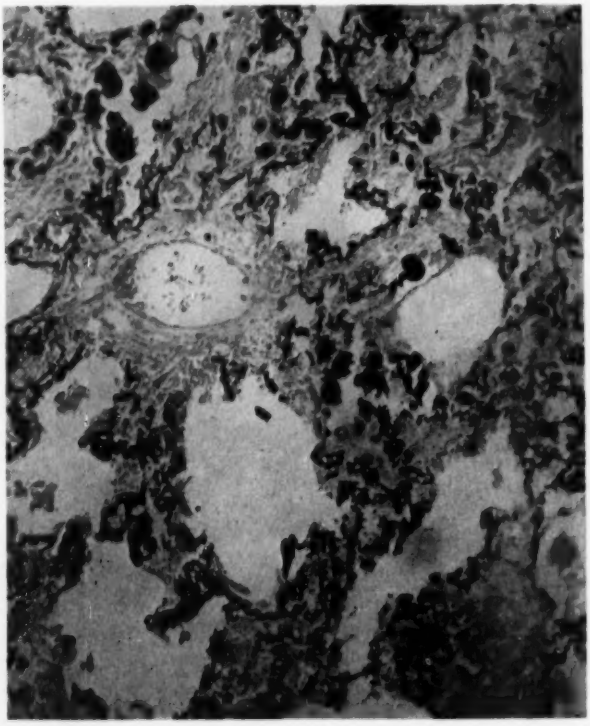

Fig. 3.—From guinea pig No. 341, exposed to kaolin dust for 12 months and then pastured for 12 more months. This is a region of interstitial fibrosis stained for calcium. Much of the calcium is concentrated in membranes which delimit the air spaces. Spheroid and ovoid bodies are present. Some of these show laminations. Von Kóssa stain; × 175.

sinuous outlines and appeared as parallel straight lines. The spaces between the straightened lamellae were often diminished, indicating a loss of smooth muscle. These changes generally began in the inner third of the media, and, while often associated with intimal proliferation, there were regions in which they were not so associated. There were other regions in which elastic lamellae were absent in the inner third of the media, whereas those of the outer two-thirds were calcified and condensed. This, of course, resulted in considerable thinning of the aortic wall, which was only partially compensated for by thickening of the intima and, focally, of the adventitia as well. Occasional small foci were found in the inner half of the media, where elastic lamellae and muscle had been replaced by a loose, fibroblastic connective tissue. Irregular calcification was present in some of these foci.

The main pulmonary artery in some of the guinea pigs showed changes identical with those present in the aorta. Smaller systemic arteries were found (renal and coronary arteries) in which the internal elastic lamina was calcified. The smooth muscle of the media was degenerated in small foci, and the intima thickened. This resulted in narrowing of the lumen in some instances.

Heart: In 12 of the 14 animals, the heart demonstrated microscopic lesions. Most of these were focal histiocytic proliferations, resembling Aschoff bodies, situated about myocardial vessels, in the endocardium, and in the epicardium. In one heart, such a cell collection was found in the ring of the aortic valve. Another interesting lesion was thickening of valve leaflets by cellular proliferation but without exudation. There was focal to extensive myocardial necrosis and calcification of the left ventricle in five hearts.

Kidneys: In all 14 guinea pigs, the kidneys were the seat of interstitial and glomer-

ular lesions. The interstitial lesions consisted of cortical fibrosis with little or no exudation. The cortical areas of fibrosis were frequently triangular, with the base of the triangle upon the capsular surface and the apex pointing toward the medulla. In several animals these areas were confluent. There was dilatation of some of the cortical tubules between the areas of fibrosis. Within the fibrotic areas, there were atrophic tubules, and Bowman's capsule was not infrequently thickened. Exudation was not often encountered but, when present, consisted of an occasional small, discrete focus of lymphocytes. Connective tissue fibers were delicate, and many plump fibrocytes gave these regions a cellular appearance.

The tubular epithelium was largely normal. It was a rare tubule that showed focal degeneration or necrosis of its lining cells.

Glomerular lesions were not rare. These consisted of focal increase in cellularity of the tufts, associated with an increase in the intercapillary acidophilic matrix. The increased cellularity of the tufts was due in part to the presence of polymorphonuclear leukocytes and in part to cell proliferation. No synechiae were found, although some of the tufts were swollen so as to practically obliterate the subcapsular space. Numerous calcific deposits were found in the interstitium, as well as within the tubules—mostly, however, within the former. Some arterioles were thickened and the lumen narrowed. The medium-sized vessels presented no significant abnormality.

No arterial, pulmonary, or cardiac lesion resembling those described in the 14 guinea pigs was found in the other 122 guinea pigs, not exposed to inorganic dust. However, the renal lesions were found in many of the controls. The most frequent finding was interstitial and tubular concretions. Small cortical scars were next in frequency. Only a rare glomerulus was found in control animals which showed an increased

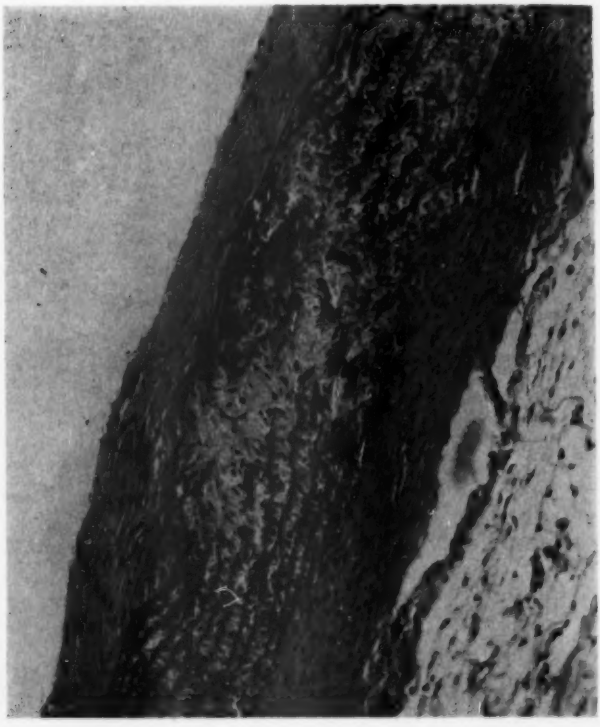

Fig. 4.—From guinea pig No. 307, exposed to glass dust for 12 months and then pastured for 7 more months. Much of the aortic media is involved by a degenerative process affecting principally the elastic lamina. These appear stretched, fragmented, and deeply basophilic. The covering intima is thickened. Hematoxylin and eosin; × 175.

cellularity of the tuft. All in all, the renal changes described as part of the disease pattern in the 14 guinea pigs did not differ in character from the renal changes seen in some of the control animals. The reported renal lesions were, however, severer and more widespread in the former than in the latter.

Comment

The pulmonary disease found in 14 out of 281 guinea pigs exposed to various dusts has the following in common with the disease described by Walford and Kaplan [1] in 12 human lungs: an interstitial fibrosis, characterized by a relatively loose connective tissue containing altered, intensely basophilic, thick elastic tissue fragments associated with multinucleated giant cells, as well as with basophilic spheroidal or globoid bodies, some of which have a laminated structure. There are, however, also differences: Whereas in the human lesions the basophilia of the altered elastic fibers and of the spheroidal bodies is associated with a positive reaction for iron and negative tests for calcium salts, in the guinea pig lungs the analogous structures are associated with positive tests for calcium salts and, except for but few instances, with negative tests for iron salts. Other significant differences include the lack (in the guinea pig) of involvement of the intrapulmonary vasculature and the absence of inclusions of asteroid or globoid bodies or of elastica fragments within the multinucleated giant cells, features which were frequently prominent in the human cases.

There are also significant findings in tissues other than lung which are important components of the disease pattern in guinea pigs, but which have not been described in the human cases of pulmonary fibrosis with altered elastic tissue. These include the involvement of aorta, the main pulmonary

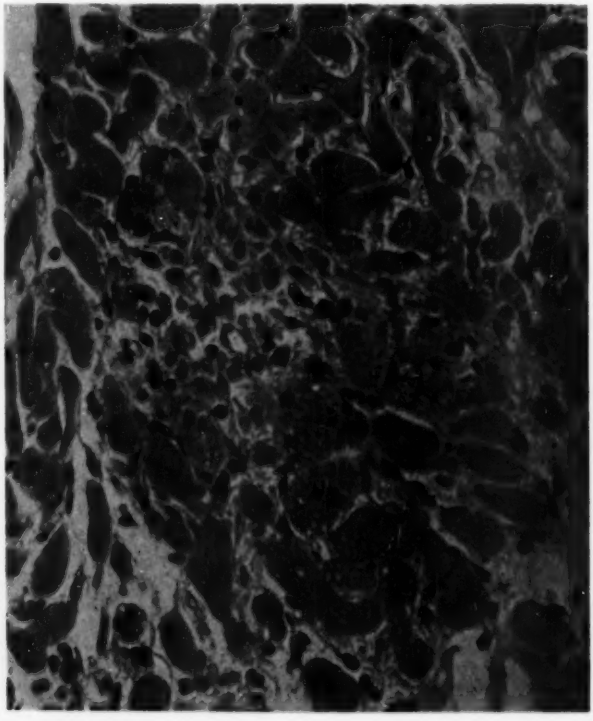

Fig. 5.—From same animal as in Figure 1. This is a collection of Anitschkow's myocytes, suggestive of Aschoff bodies, in the myocardium. Hematoxylin and eosin; ×325.

vessels, larger systemic arteries, the heart, and, to some extent, also the kidneys. This widespread involvement indicates that this disease is of a systemic character in the guinea pig. Whether or not the skin of this animal may also be involved must await future study, since, unfortunately, this tissue had been discarded.

Although the three dusts to which the 14 guinea pigs had been exposed were compounds of silicon, they were different in composition: quartz, a crystalline silicon dioxide; kaolinite, a crystalline aluminum silicate, and glass, a solid solution of amorphous silicates, of which calcium silicate was the major component. Nevertheless, they possessed one common denominator. This common denominator was an unanticipated contamination of all dusts with iron during the production of the aerosol which was subsequently blown into the inhalation chambers. As a result of this contamination, the various dusts, ordinarily colorless, were visible in the sections as golden aggregates.

Since iron is a fortuitous contaminant in the lungs of these guinea pigs, and also a result of severe vascular stasis or hemorrhage or both in the lungs of most of the 12 human cases reported, it is interesting to speculate whether iron may be an etiologic factor in the production of this disease. It seems to be the one factor common to both, human and guinea pig!

Summary

A disease of connective tissues was found in 14 guinea pigs in which an interstitial pulmonary fibrosis with degeneration of elastic tissue was the most prominent feature. Other involvement included calcific degeneration of the aorta with aneurysmal dilatation, and calcification, as well as other lesions of the heart.

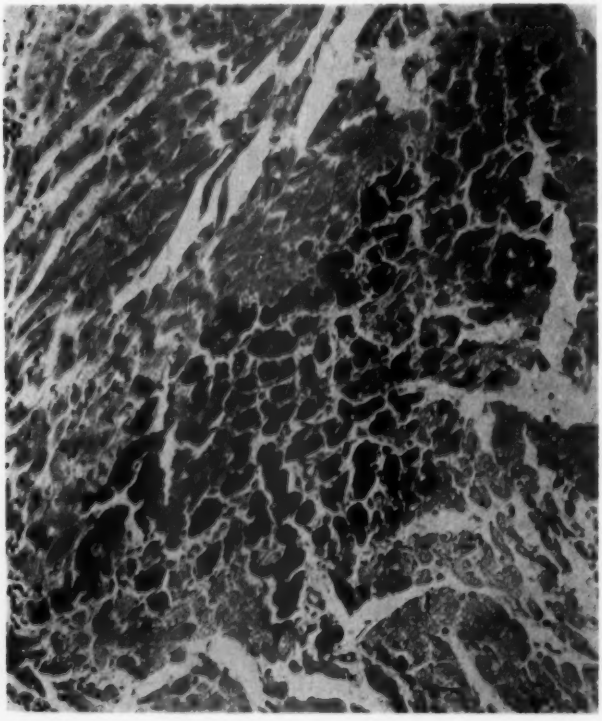

Fig. 6.—From guinea pig No. 338, which died at the age of 10 months, after an 8-month exposure to kaolin dust. One of the larger foci of myocardial necrosis and calcification is shown. A mild histiocytic proliferation is also present. Hematoxylin and eosin; × 175.

These affected 14 guinea pigs were found among a total of 281 animals that either inhaled or were given injections of one of a number of diverse dusts. None of the latter is known to be capable by itself of producing the lesions described.

Although not proved, the probability points to a common factor in the experimental technique used as the etiologic agent. A control series of 122 guinea pigs of the same sex and age range not exposed to any of the dusts in question failed to reveal evidence of this disease.

Industrial Hygiene Foundation, Mellon Institute, 4400 5th Ave.

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Pulmonary Megakaryocytes in Human Fetuses and Premature and Full-Term Infants

J. G. SHARNOFF, M.D., and VITO SCARDINO, M.D., Mount Vernon, N.Y.

This study was undertaken in order to review a larger material than was previously reported by Sharnoff and Kim,[1] in which it was demonstrated that pulmonary megakaryocytes were observed in the vascular bed of 53 premature and full-term infants, and to study the lungs of fetuses of less than five months' gestation to determine how early this phenomenon may be noted. Until the above-mentioned report was made, the concept generally held, as stated by Wintrobe,[2] was that megakaryocytes may be seen as "effete" cells with some frequency in the lungs of adult mammals. In other studies, the cells were reported observed with varying frequency in the lungs of adult humans in certain disease states by Aschoff,[3] Brill and Halpern,[4] Seebach and Kernohan,[5] and Sharnoff,[6] to cite a few. This study may also serve to substantiate further the suggestion of Sharnoff and Kim [1] that the presence of megakaryocytes in the pulmonary vascular bed is a normal phenomenon, to be seen at all ages, and may help to confirm what has been suggested by the same authors as the normal number usually seen. Confirmation of the latter would aid further in determining what may constitute the presence of increased numbers of these cells in the pulmonary blood vessels. The possible significance of this increase is discussed below.

Material and Methods

The lung sections of 103 infants of five months' gestation to term, exclusive of the 53 previously reported, as well as 21 fetuses of less than five months' gestation, were carefully scanned for the presence of megakaryocytes in their blood vessels. All lung tissues were prepared by the usual formalin fixation, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The number of megakaryocytes observed per square centimeter of lung section were recorded and averaged. The histologic appearance of these cells is described in previous reports.[1,6]

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From the Department of Pathology, Mount Vernon Hospital.

Results

The lung sections of the 103 premature and term infants, with 2 exceptions, all revealed the presence of the megakaryocytes (Fig. 1), the number averaging 2-3 per square centimeter of tissue. The lowest number was 0 to 1 cells, and the highest 7 cells, per square centimeter of tissue. The 21 fetuses, with 4 exceptions, also revealed the presence of these cells in somewhat lower number, averaging 1-2 cells per square centimeter of lung tissue (Fig. 2). The highest number seen in this group was 4. The youngest fetus in which a megakaryocyte was seen in a lung blood vessel was one of a gestation age of approximately 2½ months.

Comment

This study, and the one previously reported by Sharnoff and Kim,[1] demonstrated that megakaryocytes are found, with few exceptions, in small numbers in the vascular bed of the lungs of human fetuses and of premature and full-term infants. Including the 53 previously reported, it can now be stated that with 6 exceptions, the lungs of the 177 fetuses all disclosed the presence of these cells in small number in their capillary beds. These observations lend further support to the suggestion made in an earlier study [1] by one of us (J.G.S.) that this may be a normal phenomenon and further aids in

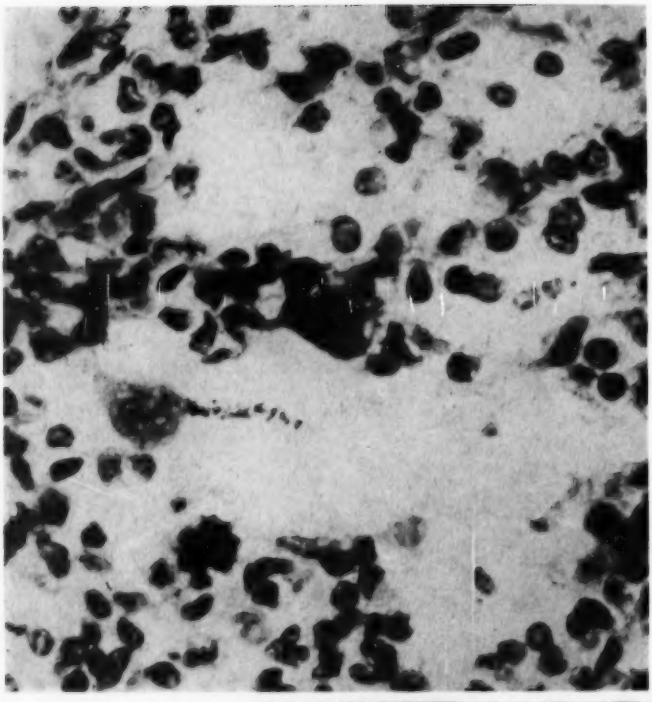

Fig. 1.—Megakaryocyte in a pulmonary blood capillary of a stillborn infant of nine months' gestation. × 827.

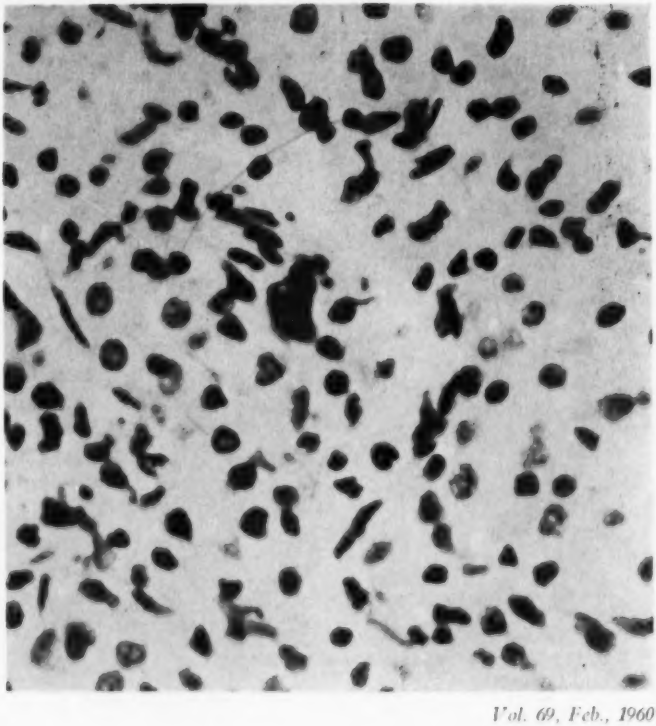

Fig. 2.—Megakaryocyte in a pulmonary blood capillary of a fetus of less than four months' gestation. × 827.

establishing what was suggested in the same report as the normal number of pulmonary megakaryocytes, namely, 2-3, generally seen in each square centimeter of lung tissue section. In establishing firmly what is the normal number of these cells seen in the lung vessels, there is thereby established what constitutes an increase in their number. An increased number of pulmonary megakaryocytes was reported observed in acute and chronic hemorrhage, thromboembolic disease, the infectious states, and thrombotic thrombocytopenic purpura.[1] This increase in pulmonary megakaryocytes, it may be reasonable to assume, can produce a sudden marked increase in circulating platelets when they disintegrate simultaneously under stressful stimuli with the increased action of the right heart ventricle. The resulting thrombocytosis may be a precipitating factor in producing blood hypercoagulability.

Summary

Pulmonary megakaryocytes were found with almost equal frequency in the capillary bed of 21 fetuses and in that of 103 premature and full-term infants.

An average of 2-3 megakaryocytes per square centimeter of lung tissue section may be seen.

This study aids in establishing more firmly what may be regarded as the normal number that may be seen in these fetuses and assists in determining what may be considered an increase in their number.

A possible explanation of the mechanism of thrombocytosis and blood hypercoagulability as a result of increased pulmonary megakaryocytes is presented.

This study was supported in part by a grant from the Westchester Heart Association.

Department of Pathology, Mount Vernon Hospital.

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Primary Aldosteronism Without Adrenal Adenomata

Report of a Case

THOMAS H. CAPERS, M.D., and GEORGE J. RACE, M.D., Dallas, Texas

Primary aldosteronism has been recognized as an entity since 1952, when Conn [1] demonstrated marked salt retention, edema, and hypertension in a patient with increased secretion of the salt-retaining aldosterone due to an adrenal cortical adenoma. Additional cases have since been reported by Evans and Milne [2] and others. With few exceptions, these have been associated with cortical adenomas. However, the cases of Van Buchem, Doorenbos, and Elings [3,4]; Bartter [5]; Holten,[6] and Kennedy et al.[7] were not associated with a tumor. In view of the apparent rarity of primary aldosteronism without cortical adenoma, an additional case is thought worthy of a report.

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From the Departments of Pathology, Veterans Administration Hospital, Dallas, and the University of Texas Southwestern Medical School.

Acting Chief, Laboratory Service, Veterans Administration Hospital, and Clinical Assistant Professor of Pathology, University of Texas Southwestern Medical School (Dr. Capers). Associate Professor of Pathology, University of Texas Southwestern Medical School (Dr. Race).

Report of Case

The patient, a 35-year-old man, a painting-company employee, entered the hospital Jan. 1, 1958, with the chief complaint of constipation of one month's duration. Three or four years before there developed gradual onset of heaviness in the left flank. This was not incapacitating but persisted during his present illness. No pain radiation was ever noticed. The heavy feeling was not accompanied by temperature elevation, chills, dysuria, or pyuria. There was a familial history of hypertension. He was seen three months prior to admission, when intravenous pyelograms were normal. One month prior to admission constipation and gassy abdominal distention developed. Findings in an upper gastrointestinal x-ray series and barium enema were normal two weeks prior to admission. The blood pressure was 150/85, but three days later it was 190/130. The urine showed some albumin, hyaline casts, and red blood cells at this time. Because of the rapidly developing hypertension, he was hospitalized for diagnostic work-up. The intravenous pyelograms were normal. Specific gravity of urine, 1.022; albumin, 300 mg/24 hr.; RBC 5-10 per high-power field; occasional hyaline casts; blood urea nitrogen, 16 mg. %. Phenolsulfonphthalein excretion was normal. Creatinine clearance, normal; sulfobromophthalein (Bromosulphalein) excretion, normal. Examination dis-

Findings in Urinalysis and Blood Studies

Date	1/1	1/3	1/4	1/8	1/9	1/12	1/15	1/17	3/20	3/20
Blood pressure	150/85		190/130	204/124						130/100
Hemoglobin, gm./%	13.8									
White blood cell count	11,500									
Urine	Alk. RBC few									
17-Ketosteroid, mg/24 hr.			18.9					25		
Serum sodium, mEq/L.			148			142	146			
Serum potassium, mEq/L.			3.6	3	3	3.2		3.5		4.4
Urea nitrogen, mg. %		16	12.9	18.4	13.7	14		10.7		
Creatinine, mg. %		1.1		1.2				1.2		
CO_2, mEq/L.		30	36		22	32		30		
Serum chlorides, mEq/L.		98	98		96	100		102		
Blood calcium, mEq/L.			5.5	4.4	4.9					
Phosphorus, mg. %			3.7	3.9	3.4					
Aldosterone urinary, μg/24 hr.				19						

OPERATION

closed Grade III eyegrounds, no cardiomegaly; but right-flank tenderness was elicited to deep palpation. Blood pressure was 204/124 in the left arm and 195/126 in the right arm; pulse 96 and respiration 18, per minute. There was no edema or gross visual defect. There had been approximately 20 lb. of weight loss over a period of three months.

Because of the loss of potassium (Table), the hypertension, and the sodium retention, the diagnosis of primary aldosteronism was made. Aldosterone assay on the patient's urine was obtained from Dr. Conn[9] through the courtesy of Dr. H. S. Seltzer. Values were 19μg. (normal, under 12μg.).

An operation for exploration of the adrenal glands for an adenoma was done. A total right adrenalectomy was performed, and nine-tenths of the left adrenal gland was removed. Biopsy specimens from both kidneys were obtained.

After the operation the patient did fairly well. The blood pressure varied from 184/120 to 170/110. A serum potassium determination was 4.4 mEq. The patient's dosage of cortisone acetate U.S.P. was reduced to 20 mg. per day. The appetite was good. Twelve days after operation the liver was found 2 fingerbreadths beneath the right costal margin. Four days later he was placed on reserpine (Serpasil), 0.25 mg. t.i.d., since the blood pressure was elevated, the diastolic range being 120 to 130.

After adrenalectomy, the patient had Addisonian crises, necessitating administration of 20 mg. of cortisone acetate daily. The blood pressure was 130/100, and the eosinophil count fluctuated between 315 and 370. Because of nausea and vomiting, an upper gastrointestinal series was performed and was normal.

Pathologic Study

Pathologic examination of the adrenals revealed the total weight, devoid of all attached fat, to be 5.54 gm. Careful sectioning of the adrenals in the sagittal plane revealed no adenoma (Fig. 1). The zona glomerulosa cells contained moderate lipid (Fig. 2). There were focal hyperchromatic and pyknotic nuclei (Fig. 3). The zone of separation between the zona glomerulosa and the zona fasciculata was indistinct. There was no apparent hyperplasia of the zona fasciculata or zona reticularis. The zona fasciculata contained large quantities of lipid.

The kidney changes (Fig. 4) were similar to those described in experimental animals subjected to potassium-deficient diets. These consisted of frequent fraying of the luminal margins of the convoluted tubules with prominent zones of vacuolation in the proximal convoluted tubules. Occasionally there were pyknotic nuclei and necrotic cells. These changes were seen in other portions of the nephron and in other parts of the collecting tubules. The vessels of arteriolar size appeared unaffected. The glomerular tufts were normally cellular, but there were a few that contained large vacuoles. A few glomeruli showed a fine deposit of a hyalin-appearing ground substance in the glomerular tufts, appearing to be located between capillary basement mem-

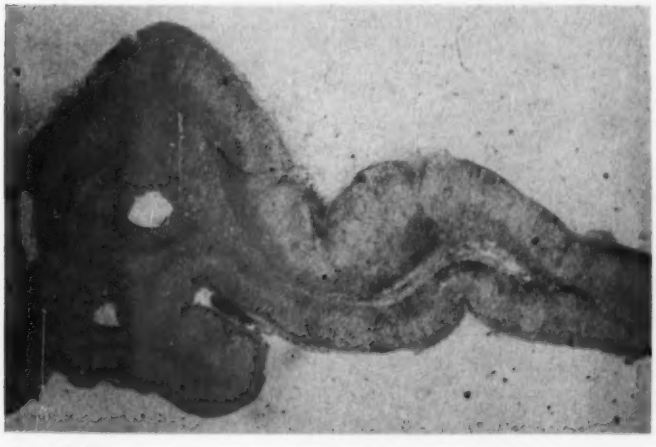

Fig. 1.—Cross section of total adrenal gland, right; low power. No evidence of an adenoma.

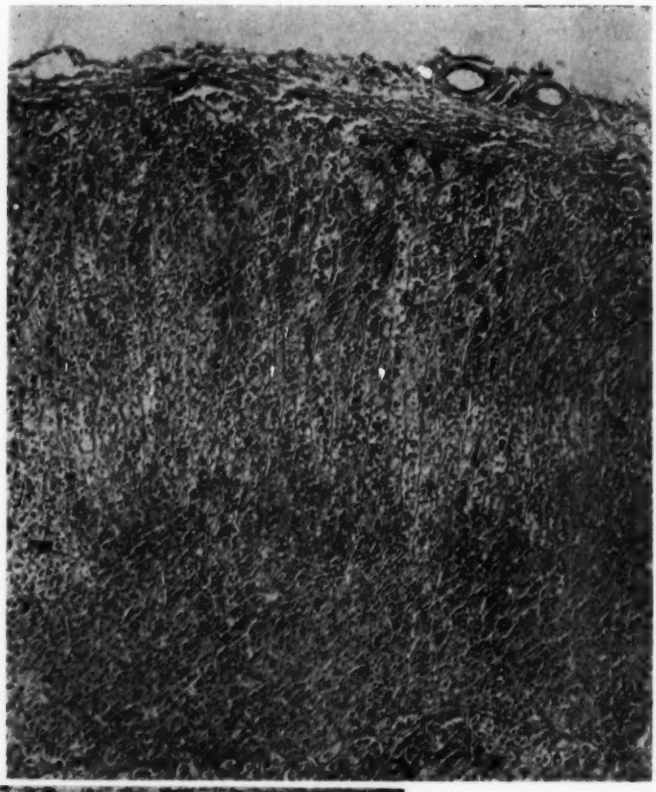

Fig. 2. — Adrenal cortex; intermediate power. Note poor distinction between the zona glomerulosa and the zona fasciculata. Also note increase of lipid in zona fasciculata, as compared with zona glomerulosa and zona reticularis. Note that the capsular arterioles are normal.

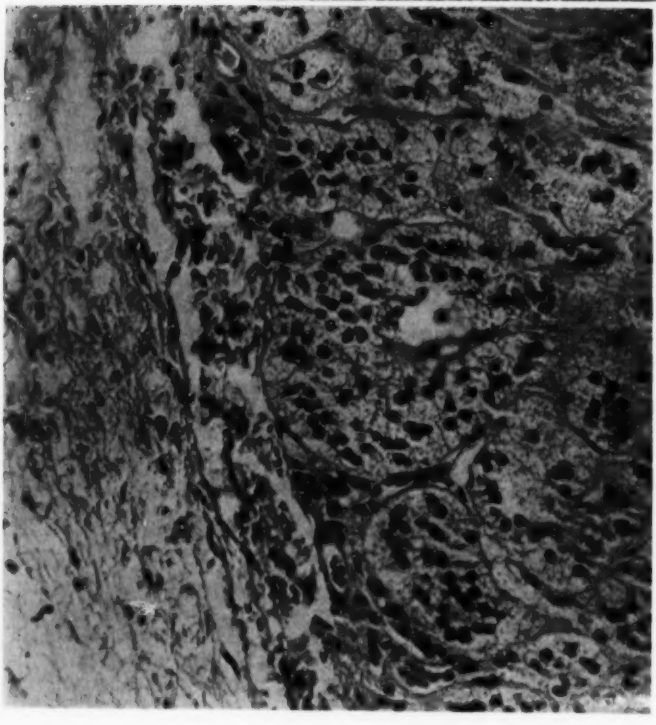

Fig. 3. — Adrenal cortex; high power; zona glomerulosa. Moderate lipid is present.

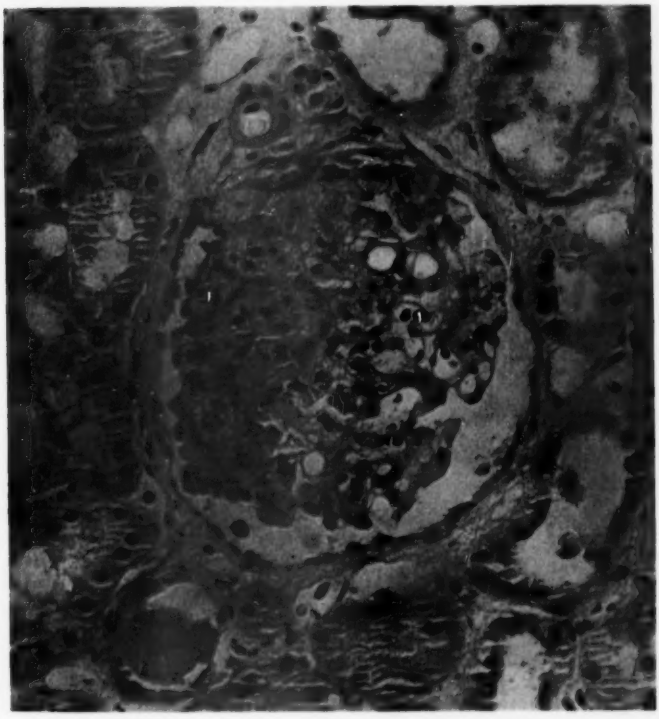

Fig. 4.—Kidney; high power. Note frayed luminal margins of convoluted tubules and vacuolation of convoluted tubule epithelium. Afferent arterioles are normal.

brane and glomerular epithelium (Tauxe, Wakim, and Baggenstoss [9]; Wyngaarden, Keitel, and Isselbacher [10]).

Comment

Conn, in 1952, demonstrated a syndrome in which there was marked salt retention, edema, and hypertension, associated with increased secretion of the salt-retaining hormone aldosterone. There are now on record 30 cases of primary aldosteronism. Almost without exception, these have been found with an adrenal cortical adenoma. However, in five cases reported the primary aldosterone syndrome was shown to be associated with adrenal cortical hyperplasia. The case which we have described is clinically typical primary aldosteronism. The patient was subjected to bilateral adrenalectomy and renal biopsy. This case was not associated with adrenal cortical adenoma or histological hyperplasia, and is therefore unusual.

Van Buchem, Doorenbos, and Elings [3,4] described some of the pathologic changes in the adrenal glands in primary aldosteronism which were associated with adenoma. In their case there were hyperplastic changes in both the zona reticularis and the zona glomerulosa of the adrenal cortex. The changes which we have seen in our case are somewhat similar but have been more pronounced in the zona fasciculata. The cortex in our case was an average of 0.93 mm. in thickness (normal 2 mm., established by Cope and Raker [11]). One gland had a cortex which was 0.85 mm. in thickness, measured by a Spencer eyepiece micrometer, calibrated against an American Optical Company stage micrometer in units of 0.01 mm. In addition, there seem to be minute atrophic changes in the tips of the zona glomerulosa in some areas.

In the renal biopsies some changes of potassium deficiency similar to those de-

scribed experimentally by Tauxe, Wakim, and Baggenstoss[9] were found.

Summary

A case of primary aldosteronism not associated with adrenal cortical adenoma is described. The patient had rapidly progressing hypertension and sustained hypokalemia, which improved following resection of one entire adrenal and 0.9 of the opposite adrenal gland. The adrenal glands were not increased in weight, showed no evidence of hyperplasia, and had no adenomata.

The authors wish to thank Dr. H. S. Seltzer, Director of Endocrinologic and Metabolic Services, Dallas Veterans Administration Hospital, for his cooperation and the use of the clinical material. Dr. Seltzer plans to report this case more extensively from the clinical point of view in the future in association with additional cases which he has collected.

Veterans Administration Hospital, 4500 S. Lancaster Rd. (16).

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Effect of Chronic Inflammation on the Epithelial Turnover of the Human Gingiva

A. S. MARWAH, D.D.S., M.S.; J. P. WEINMANN, M.D., and J. MEYER, Ph.D., Chicago

The relation between malignant growth and inflammatory reaction has been studied,[1] but the effect of inflammation on normal epithelium has not been investigated in human tissues.

In the gingiva of rats it was observed that epithelia in the vicinity of chronic inflammation had a higher mitotic rate than epithelia remote from sites of inflammation. Epithelia overlying areas of chronic inflammation, however, had a lower mitotic rate than the same region in the absence of inflammation.[2]

Chronic inflammation is common in the human gingiva, and biopsy material can readily be obtained. It was therefore possible to compare the rates of cell turnover in the presence and absence of underlying inflammation. The present study showed that the epithelium of the human gingiva responds to underlying inflammation with increased mitotic activity, unless secondary changes in the epithelial cells interfere with cell division.

Material and Methods

Biopsy specimens of human gingiva were taken in the anterior region of the mouth from 20 men aged 25 to 34 and from 24 men aged 50 to 79. The biopsy samples were obtained in the early afternoon, fixed immediately in Zenker's solution, embedded in paraffin, sectioned serially at 6μ, and stained with hematoxylin and eosin. No tangential sections were used.

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Division of Oral Pathology, University of Illinois College of Dentistry.

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The human gingiva consists of a free and an attached portion (Fig. 1). The latter is attached to the cementum of the root of the tooth and to the alveolar bone. The free portion consists of a region facing the tooth (dental surface), a crest, and a region facing the oral cavity, here designated as oral surface. The present study includes the crest region and the oral surface.

From each specimen, camera-lucida drawings at a magnification of $\times$ 100 were made of five histologic sections spaced 30μ apart. Sample regions bounded by landmarks which could be identified on the drawings and in the image under the microscope were mapped out in each section. The surface areas of the sample regions in the drawings were measured by planimetry. The surface area of tissue contained in the sample was 10^{-4} times the area measured in the drawings. The cells con-

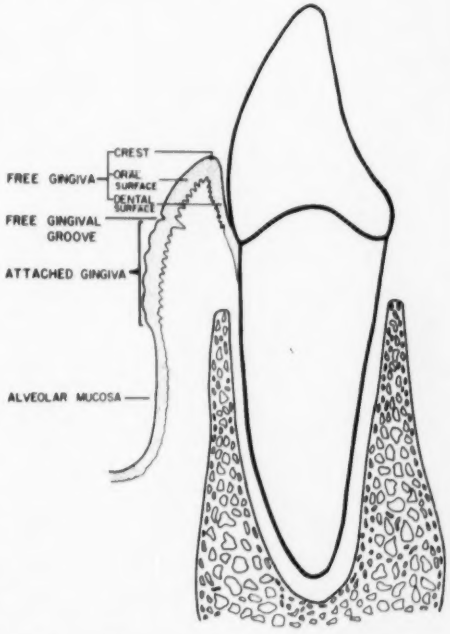

Fig. 1.—Diagram illustrating subdivisions of the human gingiva.

tained in the sample were counted under the microscope and the number of cells per unit surface computed from the cell count and the area measurement. The determinations in the five samples were averaged. This average represented the "cell density" of the specimen. The total cell population per specimen was computed from a planimetric measurement of the total surface area and from the cell density. It averaged 8,700 cells per specimen for regions of the crest and 11,300 cells for regions of the oral surface. Counts of the *cells in mitosis* contained in the included regions were made under the microscope.

Observations on Inflammation.—All specimens included appeared clinically as normal. They were classified as uninflamed or inflamed in accordance with the absence or presence of inflammatory cells in the underlying connective tissue.

In the inflamed regions, type and density of inflammatory cells in the papillary layer and in the deeper connective tissue were noted. Specimens with acute inflammation were excluded. The density of infiltration was rated by inspection on a six-point scale.

The epithelium in the inflamed specimens was examined for inter- and intracellular edema and the presence of migrating inflammatory cells. Intercellular edema was judged to be absent when the intercellular spaces were of normal width and configuration. In the present series of clinically normal gingivae, no specimen showed intercellular edema, although it was sometimes noted in the epithelium at the dental surface, which is not included in this study. Intracellular edema was judged to be present when the cytoplasm appeared fragmented and contained unstained vacuoles. From the average number of cells per unit surface the average size of the cross section of the cells were calculated. This figure includes the actual cross-sectional area of the cells, as well as that of the intercellular space. Although designated as "cell size" below, it should not be mistaken for a three-dimensional measurement of actual cell size. The degree of keratinization was examined and the presence or absence of a stratum granulosum noted.

Findings

Characteristics of Uninflamed Free Gingiva.—The *mitotic rates* in both regions of the free gingiva were found to be lower in the young than in the old group. The average mitotic index (number of dividing cells per 1,000 cells in interphase) of the crest region in the young group was 0.55; in the old group it was 0.84. The average mitotic index of the oral surface of the free gingiva was 0.49 for the young age group and 0.79 for the old age group.

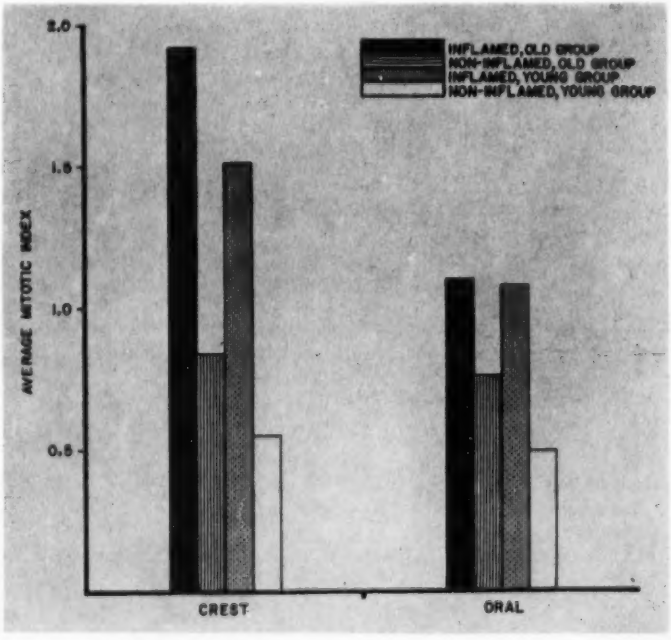

Fig. 2.—Average mitotic indices in the epithelium of crest and oral surface of the free gingiva in the presence and absence of inflammation.

The *cell density* in the crest region of the young group averaged 49 cells per $(100\mu)^2$, and that of the old group, 58 cells. At the oral surface, the young group had an average density of 50 cells; the old group, one of 59 cells.

The age difference in mitotic rate was found to be statistically significant (at the 1% level) for the oral surface, but not for the crest region, where the number of uninflamed specimens in both age groups was smaller than for the oral surface. The age difference in cell density was highly significant for both regions (below the 0.1% level).

Surface Characteristics.—All but two epithelia were entirely or in part keratinized. Full keratinization (orthokeratinization) was commoner at the oral surface; parakeratosis, or a partly nonkeratinized surface, was commoner at the crest. The two exceptions were nonkeratinized crest portions of gingiva. A granular layer was found in a third of the specimens.

Effect of Inflammation on Overlying Epithelium.—Inflammation was commoner in the old than in the young age group and in the crest than in the oral region of the gingiva. In the groups in which it was seen oftener, it tended to be severer also.

Mitotic Rate.—The averages of the mitotic rates for both regions in both age groups were between 1.5 to 3 times as high in the inflamed specimens as in uninflamed specimens (Fig. 2). However, all four subgroups contained specimens with greatly elevated mitotic indices and with indices in the normal range. Expressed as deviations from the average, the distribution of indices in *uninflamed* regions followed the peaked regular curve usually seen when mitotic rates are sampled,[3] with two-thirds of the specimens within ±50% of the average of their age and regional group, two ninths

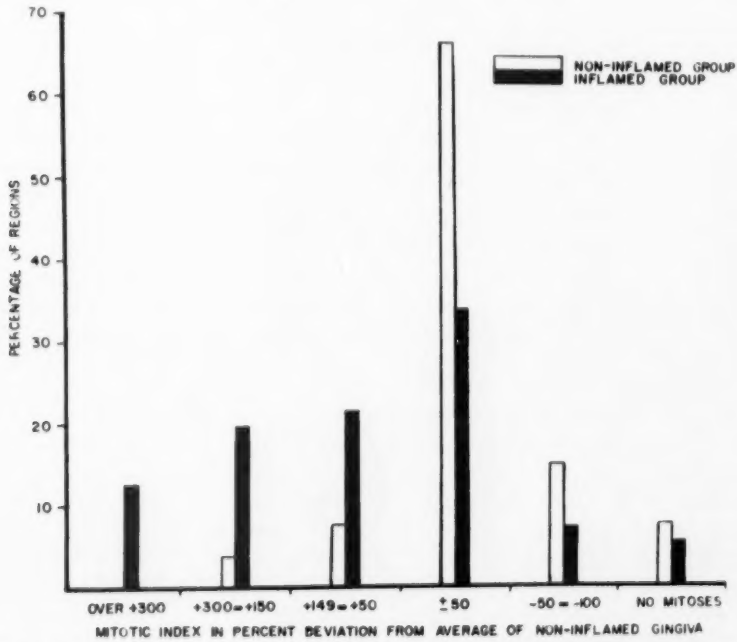

Fig. 3.—Mitotic indices in the epithelium of uninflamed and inflamed areas of the free gingiva, expressed as percentage deviations from the average mitotic index in uninflamed specimens of the same region and age.

being lower and one-ninth higher than this range (Fig. 3).

The mitotic indices in inflamed regions followed a flattened, asymmetrical curve, with one-third of the indices within ±50% of the average for uninflamed regions and one-ninth lower but five-ninths higher than this range.

It appeared from these figures that the mitotic rates in about half of the inflamed specimens were within the normal range, but that in slightly more than half the mitotic rates were elevated to figures which were not seen or were exceptional in epithelia of uninflamed regions.

Severity of Inflammation Associated with Increased and with Unchanged Mitotic Activity.—All inflamed specimens showed an inflammatory-cell infiltrate in the papillary layer, whereas infiltration of the deeper connective tissue was inconstant and dense infiltration rare. Eighty per cent of the specimens with accelerated mitotic rates, as against 30% of the group with unchanged rates, showed a dense inflammatory-cell infiltration in either the superficial or the deeper connective tissue or in both. Twenty per cent of the accelerated group, but 70% of the unchanged group, showed slight to moderate infiltration of the papillary layer and slight or no infiltration in the deeper connective tissue. Within each group, denser infiltration was associated with the higher mitotic rates.

It could be concluded that accelerated cell division in the epithelium tended to occur when the population of inflammatory cells in the underlying connective tissue was dense, whereas the mitotic rate tended to remain unaltered when the infiltration was slight. At the same time, frequent exceptions made it apparent that density of the inflammatory cells was not the only determining factor.

Epithelial Changes Associated with Accelerated and Unchanged Mitotic Activity in Inflamed Specimens.—In the absence of a complete correlation between high density of inflammatory-cell infiltration and accelerated mitotic rate, the epithelia were examined for other changes secondary to the inflammatory process.

Intracellular Edema: Intracellular edema in the epithelial cells was not common, but was noted somewhat more frequently and was slightly severer in the specimens with accelerated mitotic activity than in the specimens with unaltered mitotic activity.

Edema was quite rare in specimens having extremes of mitotic rate, i.e., those with the fastest accelerated rates and those with the lowest unaccelerated rates. Its frequency and severity were highest among the specimens with mitotic rates in the lower half of the accelerated range and in the upper half of the normal range. Moderately accelerated mitotic rates and rates in the upper range of uninflamed specimens appeared compatible with intracellular edema, but not very rapid or slow cell division.

Cell Size: The epithelial-cell sizes were expressed as deviations from the average normal for age and region (Fig. 4). The uninflamed specimens showed slight fluctuations around the average, cell size being rather constant for given regions and ages.

In all but two inflamed regions, the epithelial cell size was increased. In 10 regions it was within the upper limits of the normal range; in 44 it was outside this range. Most frequently, the size was between 14% and 18% above the average for uninflamed specimens. A cell size of 19% or more in excess of the normal was always associated with intracellular edema. Cell size was least increased in the epithelia with the higher mitotic rates.

Migrating Inflammatory Cells: A mild infiltration of the epithelium by inflammatory cells was noted in 17 specimens with accelerated mitotic activity and in 9 with unaltered mitotic activity. Six of the latter had relatively high mitotic indices.

Degree of Keratinization: Of 56 inflamed specimens, 31 were entirely unkeratinized, as compared with 2 in uninflamed specimens. The incidence of a granular layer was reduced from 1 in 3 in unin-

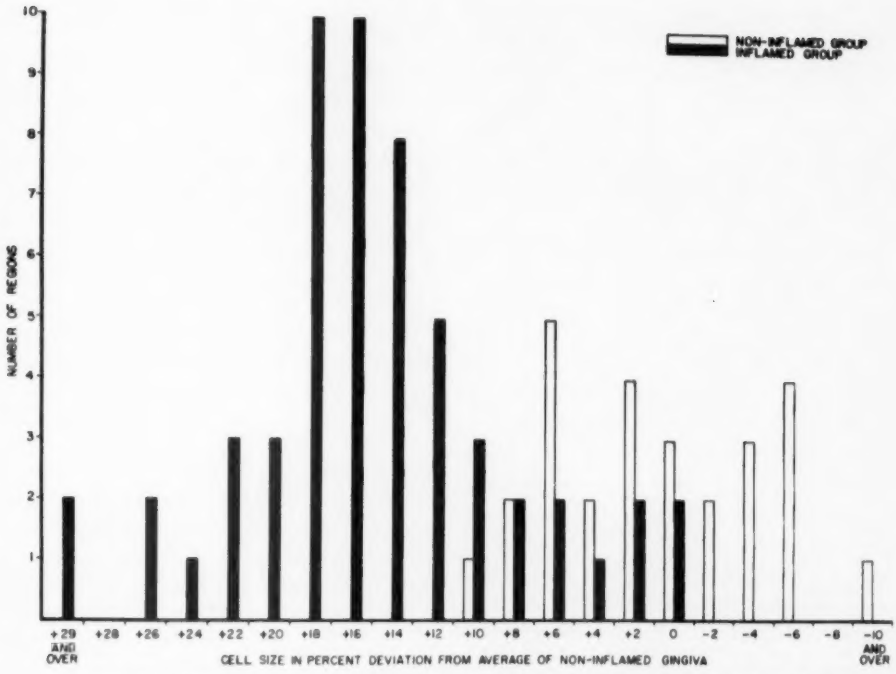

Fig. 4.—Average surface area of cells in the epithelium of uninflamed and inflamed areas of the free gingiva, expressed as percentage deviations from the average in uninflamed specimens of the same region and age.

flamed areas to 1 in 20 in inflamed areas. Full keratinization was preserved only in regions which were free of intracellular edema and of migrating inflammatory cells. Parakeratosis appeared to be slightly less sensitive to such changes.

When the mitotic rate was accelerated, keratinization was suppressed even in the absence of observable changes in the epithelium. The 15 epithelia with the highest mitotic indices were unkeratinized in 13 instances, including 9 in which intraepithelial changes were absent or slight. Fifteen epithelia with unaltered mitotic indices had fully or partly keratinized surfaces, three with the development of a granular layer. The four lowest mitotic rates were observed in fully keratinized specimens.

Comment

Characteristics of the Uninflamed Free Gingiva in Two Age Groups.—The findings in uninflamed regions of the free gingiva are an extension of a similar age study of the attached gingiva, which has been reported previously.[4] In the free, as in the attached, gingiva, the older age group shows more rapid cell turnover and smaller cell size. Literature reporting faster cell turnover with advancing age in human epidermis and in the skin of mice, and the significance of this age change with respect to cancer incidence, have been discussed in the previous publication.

Occurrence of Chronic Inflammation.—Inflammation in the gingiva differs from that in skin in that even clinically healthy regions frequently show the connective tissue infiltrated by chronic inflammatory cells. The inflammatory reaction originates at the dental surface and may spread to other regions of the gingiva. At the dental surface it is so common that some authors consider it as a physiologic process.[5] A

second difference from inflammation in the skin is the occurrence of plasma cells, which are seen in greater abundance than lymphocytes in the majority of chronic inflammations of the gingiva, in clinically normal, as well as in visibly inflamed, cases (Orban,[6] and our own unpublished analysis of consecutive biopsies).

Effect of Inflammation on the Overlying Epithelium.—Decrease of keratinization in epithelia with underlying inflammation has been noted before.[7-9] Rothman [10] has suggested that the failing keratinization of such regions might be due to accelerated surface shedding, which does not allow time for the maturing of keratin. The present study supports this view, showing that keratinization tended to be normal when cell division was not accelerated, but was repressed when the mitotic rate was high.

Cell division was found accelerated beyond the normal range of variation in about half the epithelia of inflamed specimens, whereas the other half showed rates in the normal range. A marked stimulation of cell division occurred when the accumulation of inflammatory cells in the connective tissue was dense and migrating inflammatory cells in the epithelium were common, but secondary changes in the epithelial cells slight or absent. By contrast, mitotic rates in the lower part of the normal range occurred in specimens with slight accumulation of inflammatory cells in the connective tissue, and few or no migrating inflammatory cells in the epithelium. Secondary changes in the epithelial cells were slight in this group also. Stimulation of cell division therefore seemed to depend on the density of the inflammatory cells in the connective tissue.

A moderate elevation of mitotic rates, i.e., indices between the extremes, was found when the accumulation of inflammatory cells was dense but secondary changes in the epithelial cells severe. These rates may have represented the balance between stimulation by the underlying inflammatory process and inhibition by the secondary changes in the epithelial cells which interfered with their capacity to divide.

Factors Causing Increased Mitosis.—Bullough [11] observed increased cell division in epidermal epithelia of mice in response to feeding and to the administration of glucose or of insulin at optimal glucose levels. One may conclude that one of the rate-limiting factors in epidermal cell division in the intact animal is the vascular supply of nutrients. Pinkus [12] has shown that the removal of a superficial layer of keratin provokes increased cell division in the basal layer of human epidermal epithelium, even if the bulk of the stratum corneum is left intact. Hyperemia and a mild inflammatory reaction of the underlying connective tissue precede the increase in mitotic rate. The absence of direct injury to the germinative layer in Pinkus' experiment makes it analogous to the situation in inflamed specimens of gingiva, where the injury which causes the inflammatory reaction occurs at the dental surface, but the infiltration of the connective tissue extends to the uninjured regions of the epithelium examined in this study. Increased cell division may be an unspecific response to the improved circulatory supply, as in Bullough's experiments, or a more specific response to inflammation.

The idea that lymphocytes and plasma cells act as sources of anabolites for other cells of the body has recently been revived by Ehrich,[13] and research on this concept has been reviewed by Kelsall.[1] It has been suggested that the inflammatory cells surrounding tumor transplants provide a source of raw materials and energy for which tumor and fibroblasts compete. Depending on the outcome, the tumor either benefits or is impeded by the inflammatory reaction.[1] Cancer cells have been shown to undergo increased mitosis in response to the supply of nuclear and other cell fragments.[14] If Ehrich's and Kelsall's concepts of the function of plasma cells and lymphocytes are valid, the disintegration of these cells may be responsible for the increased mitosis

seen in the gingiva. Alternatively, the inflammatory process, acting as an irritant to the epithelial cells, which eventually leads to severe secondary changes, may prior to this reach a stage at which the cells are stimulated to divide. As described above, the mitotic rate was low in epithelia with slight or no secondary changes and slight underlying infiltration, high in epithelia with slight secondary changes and dense underlying infiltration, and intermediary in epithelia with severe secondary changes and dense infiltration. These rates may be the reflection of three successive levels of irritation.

Summary

Forty-four biopsy specimens of clinically normal gingiva obtained from men 25 to 34 and 50 to 78 years of age were examined for mitotic rate, cell size, and degree of keratinization, and for presence and severity of inflammation in the connective tissue. Uninflamed specimens showed more rapid cell turnover and smaller cell size in the older than in the younger age group.

Fifty-six epithelial regions showed underlying inflammation. The effect of inflammation was the same in the younger and in the older age groups and in the crest and in the oral region of the gingiva. The epithelium of the inflamed specimens had more than 50% incidence of abnormally rapid rates of cell turnover, a decreased tendency to keratinization, and an increased cell size. Low mitotic rates were associated with slight infiltration of the connective tissue by inflammatory cells and slight secondary changes in the epithelial cells. Accelerated epithelial-cell turnover was also associated with minimal epithelial changes but denser infiltration of the connective tissue with inflammatory cells. Intermediary mitotic rates were associated with dense infiltration of the connective tissue and severe secondary changes in epithelial cells. It was concluded that at an optimal point of intensity and duration inflammation stimulates cell division in the overlying epithelium.

The authors wish to express their indebtedness to Dr. Harry S. Sicher for his critical reading of this report.

Department of Oral Pathology, University of Illinois College of Dentistry, 808 S. Wood St. (Dr. Weinmann).

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Supernumerary Kidney

Report of a Case

SAMUEL B. BURGESS, M.D., Boston

The occurrence of supernumerary kidney is rare. The first case was described by Barlet [1] in 1904, and since then cases have been reported sporadically, until 1950, when Carlson [2] summarized 50 acceptable cases and added one. Since then, several cases have been reported, some of which are acceptable, and some of which must be listed as probable but not proved.[3-8]

The difficulty regarding acceptability lies in the confusion of "double kidney" with true supernumerary kidney. These anomalies are similar entities embryologically, but are different anatomically. Double kidney, which is far commoner, is generally understood to imply a duplication of the real pelvis, ureter, and blood supply in a kidney which is anatomically a single organ. The term "supernumerary kidney" should be reserved for those cases in which there is similar duplication, but in which there is also a separation of the renal parenchyma into two organs on one side of the midline. Most authors appear to accept those cases in which the duplicated organs are attached to each other by fibrous tissue, but there are many cases in which even this fibrous tissue is lacking.

In reviewing the recent cases, it is necessary to exclude, therefore, those cases in which the sole evidence for the supernumerary kidney is radiographic, since the occurrence of two pelves and two ureters does not prove a separation of the renal substance also. Occasional reports based on intravenous pyelography will have to be accepted in view of the wide separation of the pelves, which would practically rule out the presence of a connection of renal tissue. Such a case is that reported by Begg,[3] of a woman with six functioning kidneys, demonstrated by intravenous pyelogram.

Generally speaking, however, it is wise to accept only those cases which are proved by actual anatomic exploration, either at the operating or at the autopsy table. These cases are rare indeed. A review of the files of the Mallory Institute of Pathology of the Boston City Hospital, fails to reveal a single case in more than 30,000 autopsies. The present case is unusual in that the finding was incidental to surgical exploration of the common bile duct.

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Veterans Administration Hospital, Providence, R.I., Department of Pathology, Boston University School of Medicine.

Report of Case

A 77-year-old, retired laborer entered the Veterans Administration Hospital, Providence, R.I., with a history of "flu" two weeks prior to admission. Initial symptoms of diarrhea and malaise were followed one week later by the appearance of painless, gradually increasing jaundice. There was no history of the patient having received parenteral injections; or medication such as chlorpromazine. There was no anorexia or weight loss.

The patient had been admitted twice previously at another Veterans Hospital for similar complaints, accompanied by weight loss and tarry stools, in 1944 and 1948. In each case, uneventful exploration of the common bile duct was carried out, and during the second operation the gallbladder was removed. At this time a structure interpreted as a large inflammatory node was found overlying the common bile duct.

On the present admission, physical findings were within normal limits, or were noncontributory to the present illness except for generalized icterus, extensive excoriation of the skin, and the healed scars of previous operations. Laboratory findings were consistent with a diagnosis of obstructive jaundice, but were otherwise not significant. Roentgenographic studies were noncontributory, except

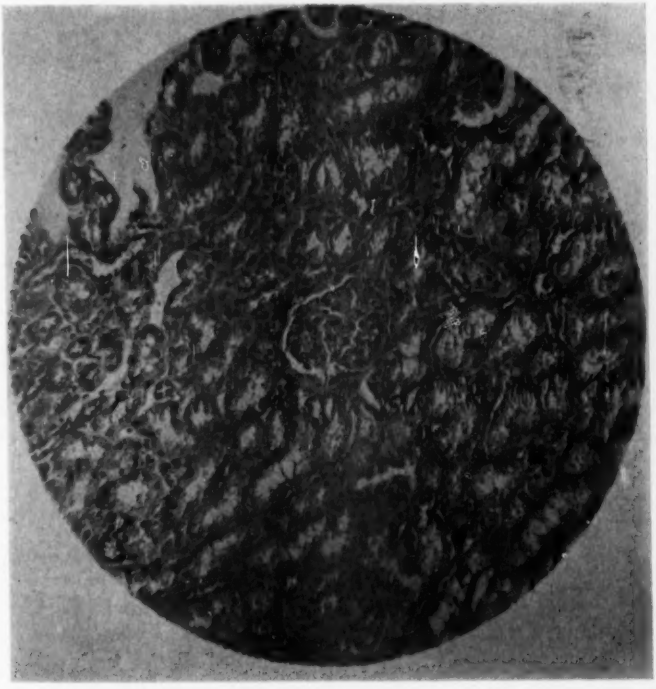

Fig. 1.—Section of the surgical specimen, showing the presence of all elements of the nephron.

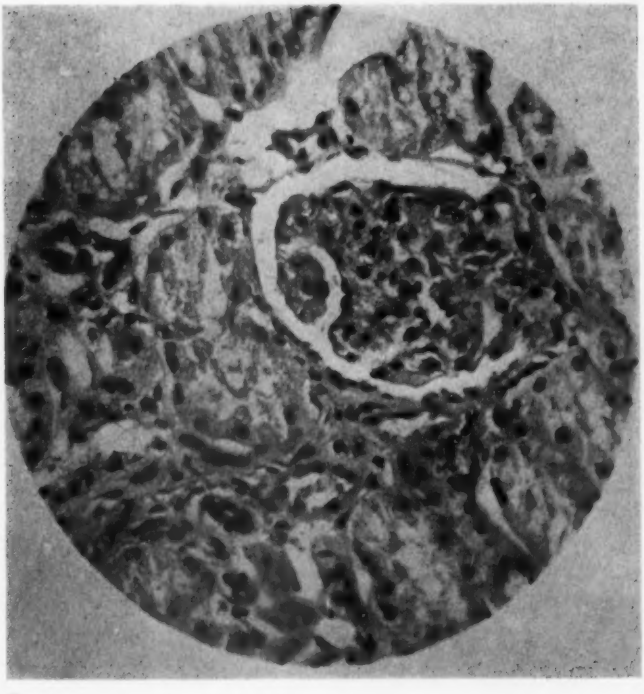

Fig. 2.—Higher-power magnification, showing arteriolosclerosis.

for intravenous cholangiography, which showed no visualization of the biliary tree.

On surgical exploration, the bile duct was found to be somewhat dilated and to contain no apparent stone or other obstruction. Again a structure thought to be an inflammatory lymph node was found close to the common bile duct at its juncture with the duodenum. This was resected. The rest of the abdominal exploration was negative, including apparently normal kidneys, identified by palpation, in their usual positions. After a stormy postoperative course, the patient recovered and was discharged. He was subsequently readmitted, and an intravenous pyelogram was obtained, which demonstrated an apparently normal kidney on each side.

Pathologic Findings.—The "lymph node" was found to be an ovoid portion of pinkish-gray tissue, measuring 2.0×1.5×0.8 cm., lying in a mass of adipose tissue. Cut surface was likewise pinkish-gray and firm, but showed a variation in markings which was not understood until microscopic sections were seen. Subsequent reexamination of the tissue showed an adherent capsule, and the unusual markings were seen to resemble the appearance of renal cortex and medulla. No structure having the appearance of renal pelvis could be identified on gross examination. Such a structure may have been present, but was lost in the initial sectioning before the true nature of the tissue was realized.

Sections were prepared in the usual manner for microscopic examination, and it was surprising to find that the "lymph node" consisted of mature renal tissue. There was moderate sclerosis of small arteries and of arterioles, and scattered glomeruli were replaced in whole, or in part, by fibrous scar. Tubules in both the cortical and the medullary portion likewise showed focal replacement by scar, and there was a very scanty, diffuse lymphocytic infiltrate. All tubular elements or levels were present. There was no evidence of tubular dilatation or of acute inflammation. A few shreds of transitional epithelium were seen at one border as a remnant of pelvic wall.

Comment

It is unfortunate that this condition was not suspected until after the preparation of the microscopic slides, as many studies might have been undertaken both prior to and at operation. An earlier diagnosis would have required clairvoyance, however, particularly in view of the rarity of the condition. It is regretted that the original sectioning of the gross specimen led to the loss of valuable information.

Study of the microscopic slides does, however, make one point clear, if only by inference. The appearance was that of functioning kidney tissue with all the necessary elements of the nephron represented, and one must suppose, therefore, that this organ was connected to the bladder in some manner, by a ureter, or hydronephrotic changes would have taken place. The moderate sclerotic changes noted seemed entirely consistent with the patient's age and general condition.

The present case is unusual in that in most previously reported cases, the smaller mass of renal tissue has been found caudal to the definitive kidney, and often in the pelvis, and in about one-fourth of the cases it is cephalad, although caudal to the adrenal gland. Here the anomalous organ was medial and ventral, and closely applied to the common bile duct. This location has not been reported previously. It would be dramatic to suggest that this patient's recurring obstructive jaundice was actually due to pressure on the bile duct by this ectopic tissue, and that a surgical cure has been effected by its removal. This is a possibility, but hardly a probability, and the cause of his jaundice remains uncertain.

In view of the relatively frequent occurrence of supernumerary organs of all sorts, it is surprising that supernumerary kidneys are so rare. Embryologically, it seems easy to visualize how this anomaly could take place; the bud of the mesonephric duct which is to become the ureter lengthens and picks up a mass of undifferentiated metanephric cells which are to form the kidney. Certainly double ureter is one of the commoner anomalies; yet the definitive renal tissue, albeit frequently with duplication of ureter and blood supply, almost always coalesces to form a single organ.

Summary

A case of supernumerary kidney, found incidental to common bile duct exploration, is described and discussed. It is reported because of the extreme rarity of this condition.

1493 Cambridge St., Cambridge 39, Mass.

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Secondary Tumors of the Heart and Pericardium in Uganda Africans

F. LOTHE, M.B., Ch.B. (Sheff.), M.R.C.P.E., and K. SOMERS, M.B., B.Ch. (W'Rand), M.R.C.P., D.C.H., Kampala, Uganda

Secondary tumors of the heart and pericardium are seen relatively commonly at necropsy, although they rarely cause clinical heart disease. It is because of their essentially pathological interest that they have evoked an extensive literature. A large number of autopsy series have been investigated with regard to the occurrence and incidence of metastatic neoplasms of the heart and pericardium. In a recent extensive survey, Prichard [1] estimated that approximately 500 instances of tumor metastasis to the heart had been recorded. A study of the literature discloses a gradually increasing incidence as the subject is more closely explored. The frequency of metastasis in known cases of malignant disease, estimated in recent surveys by various authors,[1-8] is listed in Table 1. The highest incidence is reported by Goudie [8] as 10%, by Scott and Garvin [2] as 10.9%, by DeLoach and Haynes [6] as 13.6%, and by Young and Goldman [7] as 19.1%. The last two reports do not represent the expected frequency of cardiac metastasis in general hospitals; rather, they represent frequencies of tumor reference centers with long-stay terminal cases.

The aim in this communication is to draw attention to the incidence and manifestations of metastatic tumors of the heart and pericardium in an African hospital in Uganda and to compare them with findings in Britain and America. No attempt has been made to separate neoplasms as arising in the parietal pericardium or the heart muscle or to suggest the mode of spread to the heart, for we believe that such separation, while serving a nice distinction, is often difficult to determine in the absence of unequivocal necropsy findings in a large proportion of cases. While it is possible that the parietal pericardium is mostly involved by direct extension as a result of invasion from contiguous mediastinal tissues or from lymphatic invasion, and that the heart itself

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Pathologist, Mulago Hospital, Uganda Medical Department (Dr. Lothe). Lecturer, Department of Medicine, Makerere College Medical School (Dr. Somers).

TABLE 1.—*Incidence of Secondary Tumors of the Heart and Pericardium at Necropsy*

Authors	No. of Necropsies	No. of Cases of Malignant Disease	No. of Cases of Metastatic Tumors of Heart and Pericardium	Per Cent of All Cases Malignant Disease
Scott and Garvin, [2] 1939	11,000	1,082	118	10.9
Herbut and Maisel, [3] 1942	4,050	640	35	5.4
Dimmette, [4] 1950	1,815	455	38	8.35
Prichard, [1] 1951	--	4,375	146	3.4
Willis, [5] 1952	--	500	24	5.0
DeLoach and Haynes, [6] 1953	--	980	137	13.6
Young and Goldman, [7] 1954	1,400	476 *	91	19.1
Goudie, [8] 1955	4,687	1,270	126	10.0
Lothe and Somers, 1959	6,644	377	26	6.9

* This figure excludes leukemias and brain tumors. Number inclusive of leukemia and brain tumors totaled 586.

is involved by true embolic metastasis, in our experience both parietal pericardium and heart and mediastinum are frequently involved together, and by combinations of modes of spread, in the same case. Willis [5] has given a full description of the spread of tumors to the heart and pericardium.

For the purpose of this study, leukemia must be considered as a neoplasm, as it may give rise to infiltrative myocardial or pericardial lesions.

Incidence

Mulago Hospital contains 650 beds and is situated in Kampala, the capital designate of Uganda. It is a teaching hospital of the Medical School of Makerere College, the University College of East Africa, and serves as a consultant hospital for the whole of Uganda.

An average of 470 necropsies are carried out annually at Mulago Hospital. The figure represents roughly a third of all deaths in all departments. The numbers might be higher were it not for the important factor of difficulty in obtaining consent for autopsy on religious and other grounds.

The material surveyed consists of a series of 6,644 consecutive necropsies performed at Mulago Hospital from 1947 to the middle of 1958. All the cases were African subjects belonging to a variety of tribal groups, indigenous and immigrant, living in Uganda. During the 11½-year period there were four instances of primary tumors of the heart and pericardium, of which three were malignant lymphomas.

Among 377 cases of malignant disease of all kinds throughout the body, there were 26 with macroscopic metastasis to the heart or pericardium, an incidence of 6.9%. Clearly, the true incidence is likely to be higher than this, since the instances reported were usually only those in which metastasis was evident at naked-eye examination of the outer and inner surfaces of the heart and from sections of the myocardium by incisions into each chamber. As Saphir [9] points out, foci of metastatic tumor are occasionally encountered in the myocardium if enough blocks are cut and examined histologically, even though macroscopically no tumor is present.

Pathological Findings

Distribution of Primary Site

The classification and distribution of primary sites of tumors invading the heart and pericardium are shown on Table 2.

With certain distinctions, the findings in this series are similar to the usual findings in British and American series.

Carcinoma of Bronchus and Breast.—Goudie,[8] in Britain, and Young and Goldman,[7] in the United States, have shown that a majority of metastatic tumors of the heart or pericardium are secondary to carcinoma of the bronchus. In contrast, in the Uganda series, the uncommonly low incidence of bronchial carcinoma is reflected in the number of cases in which metastasis to the heart

TABLE 2.—*Distribution of Twenty-Six Metastatic Tumors of the Heart and Pericardium*

	No. of Necropsies	No. of Cases of Metastatic Tumors of Heart and Pericardium
Carcinoma bronchus	12	4
Breast	6	1
Esophagus	7	1
Stomach	32	—
Colon and rectum	12	—
Liver	92	2
Pancreas	6	—
Kidney	6	—
Prostate	8	—
Bladder	19	—
Penis	8	—
Uterus (cervix)	3	—
Ovary	7	1
Malignant lymphoma		
Lymphosarcoma	32	6
Reticulum-cell sarcoma, jaw	7	3
Other sites	20	2
Hodgkin's disease	12	—
Skin		
Epidermoid	9	—
Malignant melanoma	3	1
Leukemia myeloid	15	1
Lymphatic	8	1
Kaposi sarcoma	3	1
Miscellaneous	50	2
	377	26

or pericardium was demonstrated. Of a total of 12 cases of bronchial carcinoma during the 11½-year period, 4 disclosed cardiac involvement. Only one of a total of six carcinomas of the breast metastasized to the heart.

Carcinoma of Liver.—As a result of surveys in Kampala, the incidence of carcinoma of the liver has been shown by Davies [10] to be very high in the African population. In this series, it was the commonest malignancy encountered at autopsy and totaled 92 out of 377 malignancies in all sites. Eight of these carcinomas were cholangiocellular and had not metastasized to the heart. The rest, comprising 84 cases, were hepatocellular carcinomas, the majority occurring in cirrhotic livers. Steiner and Davies [11] have remarked on the paucity of metastases in liver carcinoma in Uganda Africans, and it is therefore not unexpected that in only two cases were secondary tumors found in the heart and pericardium.

Malignant Lymphoma.—In 1956, on the basis of results achieved by the Kampala Cancer survey, Davies [12] expressed the view that malignant lymphoma was unusually common in the African community. This observation was certainly confirmed in our necropsy series. A total of 71 cases of malignant lymphoma were detected, and they accounted for 18.8% of all cases of malignant disease. The comparative incidence of malignant lymphoma in other series—Scott and Garvin [2] (4.1%), Herbut and Maisel [3] (4.5%), Goudie [8] (5.0%)—is listed on Table 3. The high incidence of 12.6% shown by Young and Goldman [7] does not represent the expected frequency in a general hospital, as the authors state that their hospital is a reference center for malignant lymphoma. It can therefore be said that in the Uganda series malignant lymphoma is nearly four times as common as it is in British and American series.

TABLE 3.—*Comparative Incidence of Malignant Lymphoma*

Authors	No. of Cases of Malignant Disease	No. of Cases of Malignant Lymphoma	Per Cent of Cases of Malignant Disease
Scott and Garvin, [2] 1939	1,082	44	4.1
Herbut and Maisel, [3] 1942	640	29	4.5
Young and Goldman, [7] 1955	476	60	12.6
Goudie, [8] 1955	1,270	63	5.0
Lothe and Somers, 1959	377	71	18.0

Herbut and Maisel,[3] Young and Goldman,[7] Steven,[13] and Lucia and his coworkers [14] found that of the malignant lymphomas, lymphosarcoma most frequently involved the heart. In this series of 71 cases of malignant lymphoma, 32 had lymphosarcoma, and 6 of these showed involvement of the heart. Reticulum-cell sarcoma is reported to show a somewhat less striking tendency to cardiac metastasis (Brick and Greenfield [15]; Greiner [16]). A reticulum-cell sarcoma involving the jaw, curiously common in Uganda and regarded by Burkitt [17] as the commonest malignancy seen in childhood in Uganda, was found at autopsy in seven instances in the series. Of these, three showed metastatic involvement of the heart. Only 2 of 20 cases of reticulum-cell sarcoma at various other sites had cardiac deposits. Of 12 cases of Hodgkin's disease that came to autopsy, none showed cardiac metastasis.

Kaposi Sarcoma.—A tumor seen very commonly clinically in Uganda is Kaposi sarcoma (Lothe [18]). Only three cases came to necropsy, and of them, one had secondary tumors in the heart. A case of primary Kaposi sarcoma of the heart has been reported by Gelfand [19] in a Rhodesian African.

Cardiac Metastasis.—Table 4 shows the distribution of secondary tumors in the heart and pericardium. In agreement with Willis' observations,[5] the tumors were oftener multiple than single. Thus, of 26 cases, 22 had multiple tumors and 4 had single tumors.

TABLE 4.—*Location of Metastasis of the Heart and Pericardium in Twenty-Six Cases*

Single	4
Multiple	22
Right atrium	12
Right ventricle	9
Left atrium	5
Left ventricle	10
Myocardium, site unrecorded	2
Side of heart affected	
Right only	7
Left only	3
Both	8
Myocardium, site unrecorded	2
Epicardium	19
Parietal pericardium	20
Mediastinum	15

Our numbers are too small to suggest a special susceptibility of any particular part of the heart to metastasis. If anything, the figures in Table 4 show that the right side of the heart is involved more frequently. This finding is similar to that of Prichard,[1] DeLoach and Haynes,[6] and Yater,[20] who found that the right side of the heart was more frequently involved than the left, but at variance with that of Scott and Garvin,[2] Willis,[5] and Goudie,[8] who found that all parts of the heart were equally susceptible to metastasis.

The epicardium and parietal pericardium were most frequently involved in our cases. In the majority of cases both parietal pericardium and epicardium were infiltrated with tumor tissue, and in three cases the parietal pericardium was adherent to the epicardium and the pericardial cavity was obliterated with tumor. Two of these cases were malignant lymphoma. The third was a case of bronchial carcinoma. Effusions were found in four cases at necropsy, and of these, two were hemopericardium and two were clear effusions. The high incidence of mediastinal involvement has been noted by many observers, and this has been stressed particularly by Young and Goldman[7] and Lymburner.[21] Filling of mediastinal lymph nodes and mediastinal tissue with tumor is likely to block lymphatic channels draining the heart and allows retrograde extension through subepicardial lymphatic vessels. In this series the mediastinum was significantly involved in 14 cases with primary tumor in various sites, including bronchus (3 cases), liver (2 cases), and malignant melanoma (2 cases).

Metastasis in Other Organs.—In all 26 cases there were either extensive generalized metastasis or widespread intrathoracic dissemination of tumor.

Clinical Findings

It is difficult to correlate clinical effects of secondary growths with cardiac function. Clinical attention is seldom directed to the heart in cases in which the primary tumor overshadows any features of cardiac involvement. Specific symptoms referable to the heart may be submerged in the general cachexia and distress of advanced intrathoracic malignant disease. Disorders of rhythm and conduction escape recognition because electrocardiographic and other special investigations are seldom carried out in such cases. On the other hand, in patients who during life have exhibited little or no evidence of cardiac failure or disturbed rhythm, necropsy may reveal extensive replacement of the myocardium or invasion of the chambers by tumor.

These remarks, shared by Willis[5] and Goudie,[8] are certainly true of this study. From a perusal of clinical notes, it emerges that, while cardiac metastasis was suspected in eight cases from the clinical features of dyspnea (one case), superior vena caval obstruction (five cases), and the radiological appearance of an enlarged heart shadow (two cases), none was definitely diagnosed during life.

In his excellent review of 500 published cases of secondary tumors, Prichard[1] reported that in only 20 had the diagnosis been made ante mortem, usually on the development of cardiac arrhythmia in a patient known to have widespread malignant disease. Nonetheless, as stressed by Yater,[20] the onset of cardiac symptoms or findings of cardiac disease without apparent cause in a patient with known malignancy are highly suggestive of cardiac involvement by tumor.

In any case, a certain diagnosis is of more than academic importance, for a remission of a metastatic tumor and relief of disablement may be produced as a result of treatment.

Summary

In a series of 6,644 consecutive autopsies performed at Mulago Hospital during the past 11½ years, a total of 377 cases of malignant disease, of which a high proportion were malignant lymphomas, were observed. Secondary tumors of the heart or pericardium are not rare in patients dying of malignant disease in Uganda, and the over-all incidence is similar to that reported in Western communities. The heart and pericardium were involved by metastatic tumor in 26 cases, an incidence of 6.9%. Malignant lymphoma accounted for the majority of secondary tumors of the heart and pericardium. Three common malignancies occurring in Uganda—liver carcinoma, reticulum-cell sarcoma of jaw, and Kaposi sarcoma—are shown to produce cardiac metastasis. Carcinomas of the bronchus and of the breast are uncommon causes of cardiac metastasis because their occurrence is uncommon in Uganda. Clinical manifestations of these tumors and ante mortem diagnosis are unusual except on careful interpretation of cardiac symptoms or signs in patients with malignant disease.

Illustrative Case Summaries

CASE 1.—*Primary mediastinal lymphosarcoma with multiple intrathoracic metastasis.*

Clinical Findings

A Ganda man, aged 37, was admitted with a history of cough, dyspnea, and hoarseness for four

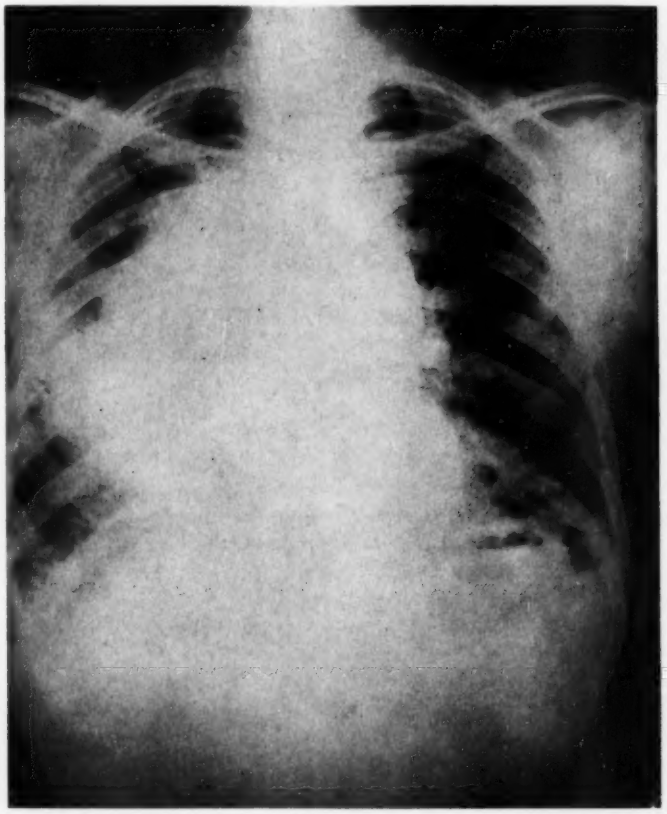

Fig. 1 (Case 1).—Lymphosarcoma. Roentgenogram of chest showing widened superior mediastinum and large rounded shadow at right hilus, continuous with heart shadow.

weeks. On examination he was found to be extremely weak and showed signs of superior vena caval obstruction. Heart size and sounds were normal. B.P. was 120/75. There were no signs of heart failure. Percussion note was impaired, and breath sounds were diminished throughout the right lung field. Blood investigations showed hemoglobin 96% (14.2 gm./100 ml.); WBC 8,000/cu. mm. (neutrophils 37%, lymphocytes 60%, eosinophils 1%, monocytes 2%). Roentgenogram of the chest (Fig. 1) showed widening of the superior mediastinum and a large rounded shadow at the right hilus, continuous with the heart shadow. Small, patchy opacities were present in the left lung base.

Death took place suddenly within a few days of admission.

Necropsy

Externally, the face, upper limbs, and upper chest were edematous. The jugular veins and veins in the upper limbs were markedly engorged.

The sternum and anterior mediastinum were infiltrated with white solid tumor nodules with necrotic, soft centers. The upper and middle lobes of the right lung were replaced by tumor. Tumor nodules were scattered in the left lung and both pleura.

The heart and aortic arch were encased in a tumor mass. Pericardial cavity was obliterated with adherent tumor deposits. Several nodules on the epicardium had infiltrated into the heart muscle.

Microscopic examination of tumor tissue showed lymphosarcoma of uniform small round-cell type (Fig. 2).

Comment

This patient presented with superior vena caval obstruction and pulmonary symptoms. A mediastinal tumor was diagnosed on radiological findings. Necropsy showed lymphosarcoma with involvement of the heart and pericardium.

CASE 2.—*Hepatocellular carcinoma in a cirrhotic liver with multiple metastasis.*

Clinical Findings

A Zinja man, aged 40, was admitted with a history of pain in the right hypochondrium and

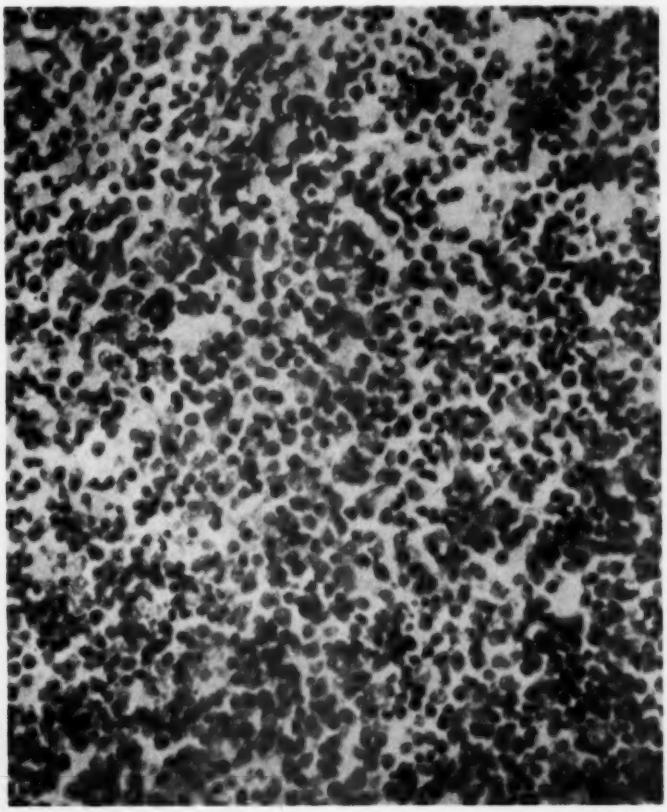

Fig. 2 (Case 1.)—Lymphosarcoma. Section showing uniform small-round-cell lymphosarcoma. Hematoxylin and eosin; × 100.

enlarged lymph nodes on the right side of the neck. He also had paraplegia of recent onset. Examination revealed an emaciated, jaundiced patient with signs of superior vena caval obstruction.

Veins over the abdominal wall were also engorged. The liver edge was coarsely nodular.

The patient died suddenly, before investigations could be completed.

Necropsy

The patient was emaciated, with a tinge of jaundice.

The right lobe of the liver was filled by a yellow, necrotic mass of tumor. Scattered nodules were present throughout the liver, which was grossly cirrhotic. Mesenteric and mediastinal lymph nodes were infiltrated with tumor. Multiple nodules were scattered in both lungs.

The epicardium was invaded by tumor nodules, involving particularly the left atrium and left ventricle.

A mass of tumor tissue had invaded the lower thoracic spine and caused pressure necrosis of the cord.

Histologic examination of the tumor showed hepatocellular carcinoma.

Comment

This patient presented a malignancy of the liver. Associated features of superior vena caval obstruction were shown at necropsy to be due to a disseminated hepatocellular carcinoma. The epicardium and heart muscle were involved by tumor deposits.

CASE 3.—*Carcinoma of bronchus with intrathoracic spread.*

Clinical Findings

A male Teso, aged 28, was admitted with a history of a dry, distressing cough and dyspnea for one month.

Examination showed a very ill patient with signs of fluid in the right chest. The heart sounds were faint and distant. Roentgenogram of chest (Fig. 3), however, showed a large pericardial effusion and fluid in the right pleural cavity. An

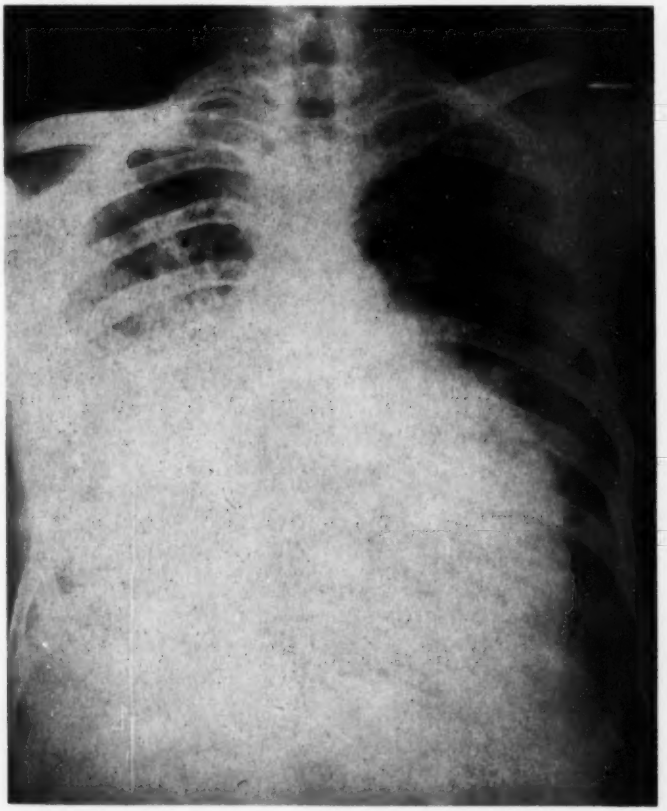

Fig. 3 (Case 3.)—Carcinoma of bronchus. Roentgenogram of chest showing pericardial and right pleural effusions.

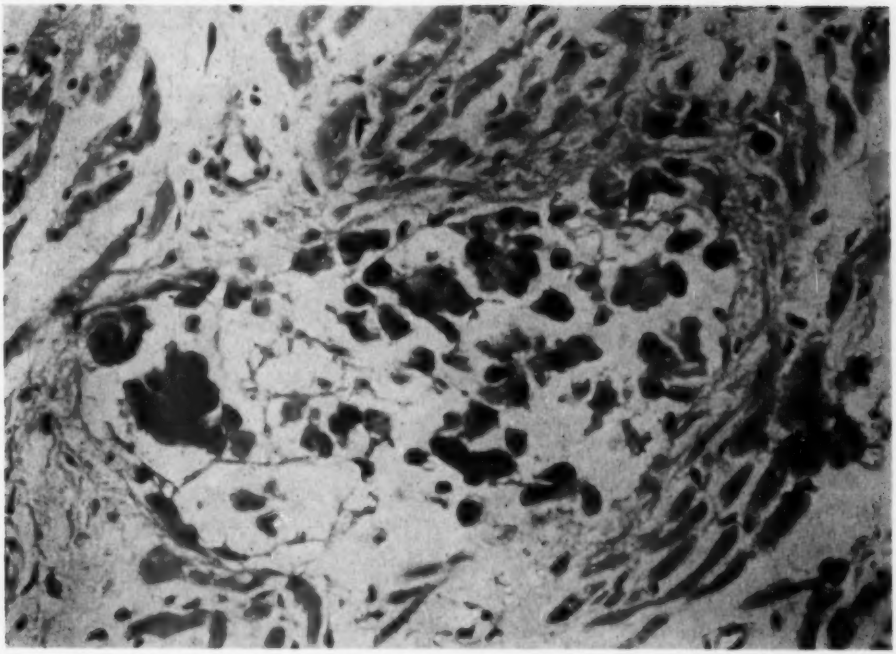

Fig. 4. (Case 3).—Carcinoma of bronchus. Section of heart muscle showing metastasis of adenocarcinoma. Hematoxylin and eosin; reduced to 94% of mag. × 100.

immediate pericardiocentesis revealed a hemopericardium; the fluid rapidly reaccumulated in a few days, followed by death in cardiac tamponade.

Necropsy

The trachea was deviated slightly to the left by a moderate-sized straw-colored right pleural effusion. The right lung on section was solid and airless with numerous streaks of tumor tissue. Both hilar lymph nodes were infiltrated with tumor.

The pericardium contained 800 ml. of blood-stained fluid overlying widespread epicardial tumor deposits. The myocardium was infiltrated with streaks of tumor tissue.

Microscopic examination revealed adenocarcinoma of bronchus (Fig. 4).

Comment

This case presented with pleural effusion and hemopericardium due to secondary tumor invasion from a carcinoma of bronchus.

CASE 4.—*Reticulum-cell sarcoma of jaw with multiple secondary tumors.**

Clinical Findings

A male Samya child, aged 7 years, was admitted with a history of swelling of the face and of the left testis for three months. The right lower jaw had also increased considerably in size in the past three weeks. He had lost much weight and was admitted in a cachectic state.

Examination showed a large swelling involving the anterior end of the right side of the lower jaw, displacing and lowering the teeth. Another swelling was seen to bulge from the left half of the maxilla through the roof of the mouth.

Roentgenograms of the skull showed cystic areas in the left half of the maxilla and an opacity of the right maxillary antrum. A biopsy of the lower jaw showed a small-round-cell sarcoma. A roentgenogram of the chest showed a generalized enlargement of the heart shadow, consistent with a pericardial involvement by tumor.

His condition deteriorated rapidly and he died suddenly, presumably of cardiac tamponade.

Necropsy

The right and left maxillary antra and both halves of the lower jaw were filled with soft tumor-tissue masses.

The heart and pericardium were encased in a mass of tumor. The pericardial cavity was obliterated by tumor infiltration. On section of the heart, nodules of tumors were found in the right atrium and right and left ventricles (Fig. 5).

* We are grateful to Mr. Burkitt for details of this case.

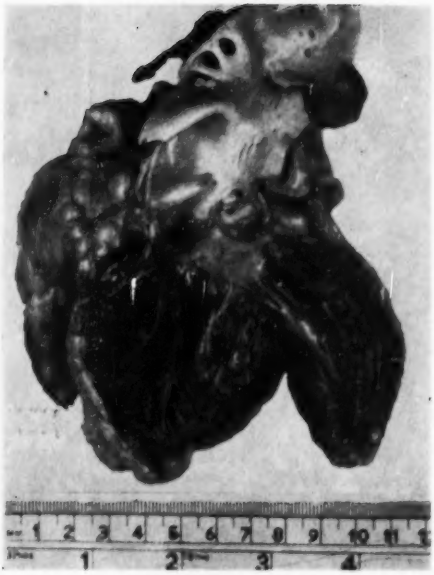

Fig. 5 (Case 4.)—Reticulum-cell sarcoma of jaw. Specimen showing tumor mass encasing heart.

Both kidneys, thyroid, and left testis were the seat of secondary tumors.

Histological sections of the tumor showed a reticulum-cell sarcoma (Fig. 6).

Comment

This case was one of reticulum-cell sarcoma of the jaw associated with multiple tumor deposits. The localization of the tumor in all four jaw quadrants separately suggested a multicentric origin.

This survey was initiated on the suggestion of Prof. J. N. P. Davies, and we are grateful to him and to Professor Williams for encouragement and advice. We are also grateful to members of the Clinical Divisions and the Department of Pathology of Mulago Hospital and the Kampala Cancer Registry for access to notes and records, and to the Director of Medical Services, Uganda, for facilities at Mulago Hospital.

P.O. Box 2072, Kampala, Uganda.

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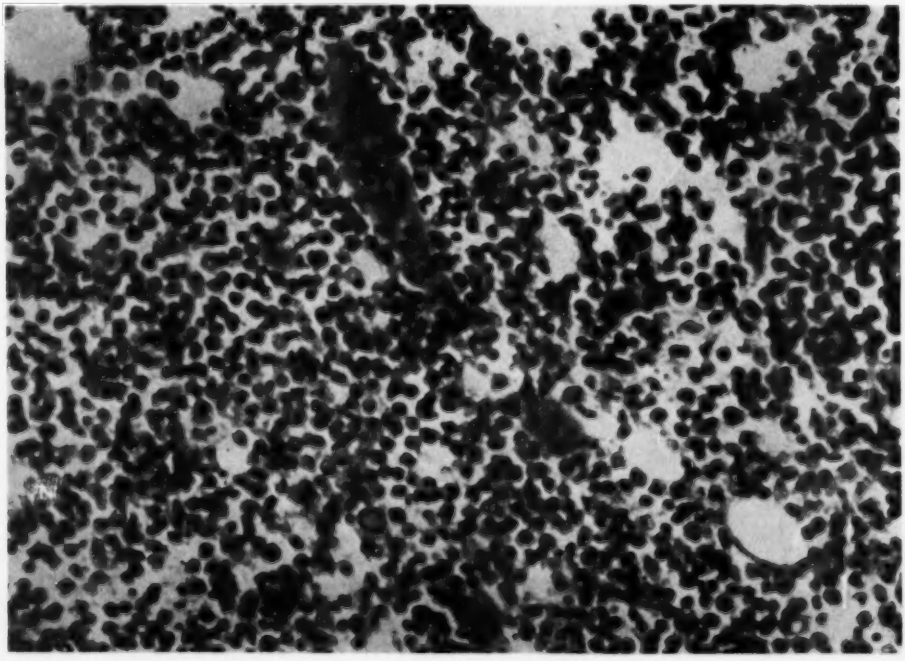
Fig. 6. (Case 4).—Reticulum-cell sarcoma of jaw. Section of tumor tissue. Hematoxylin and eosin; reduced to 94% of mag. × 100.



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Recovery from Dietary Cirrhosis of the Liver in the Rat

Changes in Hepatic Collagen and in Microscopic Appearance

ARTHUR J. PATEK Jr., M.D.; DONALD E. OKEN, M.D.; ARTHUR SAKAMOTO, M.D.; NANCY de FRITSCH, B.S., and MARGARET BEVANS, M.D., New York

Although there have been many reports on the production of dietary cirrhosis in the rat, relatively few studies have been directed at the recovery phase of the disease. In earlier experiments reported from this laboratory and from others [1-3] histological evaluation alone was employed. With few exceptions,[4] the recovery phase was observed for only four or five months. In livers with less severe degrees of cirrhosis there appeared to be resorption of connective tissue after animals were fed diets rich in protein or in lipotropic substances, such as choline or methionine. However, it was not possible to ascertain whether the disappearance of connective tissue was real or apparent, owing to thinning and stretching by regenerating liver cells.

In a more recent study [5] reported from this laboratory, both histological examinations and gravimetric assays of collagen were carried out. Rats were fed a 4% casein diet for four months, at the end of which time about 75% exhibited cirrhosis on biopsy. The cirrhotic animals were then fed therapeutic diets containing 30% casein or amino acid mixtures equivalent to 30% or 50% casein as the source of protein. Their livers showed a sharp decrease in the content of collagen when compared with the untreated controls fed the 4% casein diet.

The present experiment is an extension of the above studies on the recovery from dietary cirrhosis. 1. The period of dietary treatment is increased from 4 months to at least 10 months, with the aim of bringing about more complete recovery. 2. The therapeutic diet employed is a commercial Chow in place of the casein mixtures. 3. As in the earlier studies, histological comparison of livers is made from sections obtained at biopsy preceding therapy and at autopsy after 10 months of dietary treatment. 4. Collagen is estimated on the basis of hydroxyproline determinations, according to the method of Neuman and Logan.[7] This procedure is simple and accurate, and appears to be more practicable than the method of Lowry et al.,[8] previously employed.

Plan

Material.—Male albino rats of the Sprague-Dawley strain, approximately 6 weeks old and weighing 100 to 150 gm., were housed in separate cages. Animals serving as normal controls were fed a commercial Chow diet.* Test animals were fed a cirrhosis-producing diet which contained 4% casein as the source of protein (Table 1). The colony was divided into four experimental groups, according to the scheme shown in Table 2.

Group 1 was fed the 4% casein diet for six months. Biopsy of the liver was then carried out at laparotomy. Animals with demonstrable cir-

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From the Research Service, First (Columbia University) Division, Goldwater Memorial Hospital, and the Department of Medicine, Columbia University College of Physicians and Surgeons.

* Purina Laboratory Chow (Ralston) is derived from natural foods, chiefly meat, fish, grain, yeast. Its approximate chemical composition is as follows: crude protein 25 gm.; fat 5 gm.; fiber 6 gm.; nitrogen-free extract 47 gm.; ash 7 gm.; minerals, salts, etc. 10 gm.

TABLE 1.—*Composition of Cirrhosis-Producing Diet*

	Per Cent
Vitamin-free casein	4.0
L-cystine	0.5
Cottonseed oil	5.0
Salt mixture *	3.0
Cornstarch	86.5
Vitamin mixture †	1.0

* Hubbell, R. B.; Mendel, L. B., and Wakeman, A. J.: New Salt Mixture for Use in Experimental Diets, J. Nutrition 14:273, 1937.

† Vitaminized cornstarch, of which 1 gm. supplied the following in milligrams: thiamine HCl, 1.25; riboflavin, 0.625; calcium pantothenate, 0.625; pyridoxine HCl, 0.25; nicotinic acid, 12.5; aminobenzoic acid, 10.0; folic acid, 0.05; menadione U.S.P., 0.1; alpha tocopherol 4.0. Vitamin A (Oleum Percomorphum), 750 U.S.P. units, and vitamin D 108, U.S.P. units, were added for 100 gm. diet in 200 mg. cottonseed oil.

TABLE 2.—*Scheme of Experiment—Distribution of Animals*

Group	Fore Period—6 mo. No. of Rats	Fore Period—6 mo. Diet	At 6 Mo.	After Period—10 Mo., Diet
1 (cirrhotic)	53	4% casein	Biopsied	Chow
2 (normal)	17	Chow	Biopsied	Chow
3 (cirrhotic)	20	4% casein	Killed	--
4 (normal)	10	Chow	Killed	--

rhosis, 53 in number, were placed on the commercial Chow * diet for the ensuing 10 months and then killed. Their livers were analyzed histologically and chemically for collagen concentration.

Group 2 consisted of 17 rats receiving the Chow diet for six months, at which time their livers were biopsied. These rats were fed the same Chow diet for 10 more months and then killed. Their livers were analyzed in the manner described for Group 1.

Group 3 consisted of 20 rats with demonstrable cirrhosis killed after six months on the 4% casein diet. They served as untreated cirrhotic animals.

Group 4 comprised 10 rats which were killed after six months of feeding on the Chow diet. They served as controls for Group 3.

In addition to the above groups, 58 rats were eliminated from the study. These included 36 which failed to develop cirrhosis after six months on the 4% casein diet. They were considered unsuitable for this experiment. There were 8 rats that died during the fore period of six months and 14 that died later, in the course of the study, or whose tissues were lost or spoiled. In some instances death was due to intercurrent infection, such as pneumonia.

Methods.—The cirrhosis-producing diet (Table 1), based upon one devised by Daft, Sebrell, Lillie,* is the diet we have employed in earlier studies. The Chow diet, serving as a therapeutic diet, has the composition shown in the asterisk Footnote. On the latter diet rats grew normally and remained healthy for the 16-month period of observation. All animals were fed ad libitum. They were weighed at biweekly intervals.

Liver biopsies were performed under ether anesthesia. A midline longitudinal incision was made in the epigastrium. A wedge-shaped piece of liver tissue, about 0.5 cm. at the base, was cut from the presenting lobe. The defect was filled with absorbable gelatin sponge U.S.P. (Gelfoam) and the wound closed either with interrupted silk sutures or with metal clips.

Rats were killed by exsanguination under light ether anesthesia. Sections of liver were removed for histologic study. The remainder of the liver was wrapped in aluminum foil, frozen, and stored at —30 C, until used for chemical analysis.

Specimens were fixed in Zenker's solution and in 10% formaldehyde. Routine paraffin sections were stained with hematoxylin and eosin. Trichrome stains were employed for the better definition of connective tissues. Frozen sections stained for fat with oil red O and other special stains were used when indicated. Connective-tissue proliferation was graded on a scale of 1+ to 4+ by two observers. One plus (1+) indicated minimal increase of connective tissue about the portal triads and central veins; 2+, extension of connective tissue into the lobule but no disruption of the liver cords; 3+, distortion of liver architecture, due to intra- and perilobular connective-tissue proliferation, and 4+ maximal distortion. Although estimation was made of fat, necrosis, bile-duct proliferation, ceroid deposits, and inflammatory cellular reaction, these were not included in the grading.

Collagen Determination.—Portions of tissue were taken from the periphery of one lobe of each liver. Tissue adjacent to the porta hepatis was avoided and the liver capsule carefully excluded. Each sample weighed approximately 3 gm. Samples of 12 to 18 livers were analyzed concurrently, each group containing livers randomly selected.

Each sample was washed in water and minced into a paste, to which 40 ml. of acetone was added and stirred intermittently for six hours. The acetone was decanted and the sample stirred with 40 ml. of fresh acetone for six hours. A third acetone extraction was performed, after drying and grinding the sample to powder. This was followed by extraction with 40 ml. of anhydrous ether for two six-hour periods. The powder was then dried to constant weight in an oven at 100 C. At this time it was considered to be fat-free and dry.

Precisely 50 mg. of each fat-free, dry liver powder was weighed into a Pyrex test tube. Soluble protein, which might interfere with subsequent analysis, was removed by stirring the powder with 20% urea solution for one hour. The urea solution was poured off after centrifugation, and the samples washed twice with distilled water. Care was taken to avoid losing any of the insoluble tissue during these procedures.

The collagen concentration of the sample was calculated from the concentration of hydroxyproline, an amino acid found in constant proportion in collagen but absent from cellular protein. After hydrolysis of the sample in 1 ml. of 6 N HCl for 16 hours at 110 C, hydroxyproline concentrations were determined colorimetrically by the technique of Neuman and Logan.[7] Collagen values were obtained by multiplying the hydroxyproline concentrations by 7.46 (collagen contains 13.4% hydroxyproline)[7] and were expressed as milligrams of collagen per 100 mg. of fat-free, dry liver tissue.

Experimental Findings

Figure 1 presents the average growth curves of the groups observed for 16 months. During the fore period, rats on the 4% casein diet grew slowly; their fur was shaggy, and they seemed irritable. When the 4% casein diet was replaced by the Chow diet, their growth rate rapidly accelerated, and their fur and general nutrition were much improved. As they grew older and heavier, the animals became relatively sluggish.

The control animals fed the Chow diet appeared to be in normal health throughout the experiment. Liver sections obtained at the time of biopsy and at autopsy were normal, histologically.

The development of pathologic hepatic changes in animals fed protein-deficient diets

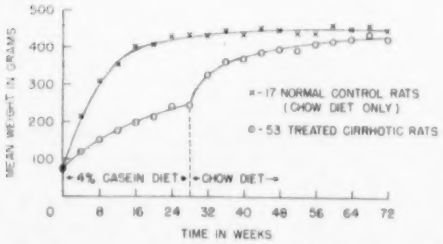


Fig. 1.—Growth curves of animals observed over a 16-month period (Groups 1 and 2).

TABLE 3.—*Histological Severity of Cirrhosis*

Groups	Degrees of Cirrhosis: 0 ±	1+	2+	3+	4+
1 Cirrhotics (53) before therapy (6 mo.)	0	5	20	24	4
Cirrhotics (53) after therapy (16 mo.)	45	4	4	0	0
2 Normals (17) at 16 mo.	17	--	--	--	--
3 Cirrhotics (20) no therapy (6 mo.)	0	3	7	5	5
4 Normals (10) at 6 mo.	10	--	--	--	--

has been described in detail by others,[9-11] as well as in our earlier reports.[3,5] The lesions found at biopsy, after six months on the 4% casein diet, were similar to those previously encountered. With few exceptions the liver showed extensive fatty infiltration. Many livers exhibited fatty cysts. There was considerable variation in size and staining quality of the liver cells. There was little or no evidence of necrosis. Moderate cellular infiltration with lymphoid cells was seen. There were varying degrees of connective-tissue proliferation and distortion of hepatic structure. Roughly 30% of the animals had failed to develop connective-tissue changes at the time of biopsy, although most of these had fatty livers.

The reparative changes produced by dietary treatment were impressive (Table 3). The restoration of normal hepatic structure, the almost complete disappearance of excessive connective tissue and lymphocytosis, and the normal-appearing parenchymal cells were in sharp contrast to the findings obtained at biopsy preceding therapy. Livers which had shown moderate degrees (1+, 2+) of fibrosis at biopsy appeared to be quite normal on microscopic section at autopsy. Several that had shown advanced cirrhosis (3+, 4+) at the time of biopsy also appeared normal at autopsy. However, the majority of animals with advanced cirrhosis at 6 months still showed a few fine scars in the liver after 10 months on the Chow diet. Usually this consisted of increased connective tissue markings about the veins (Figs. 2, 3, and 4).

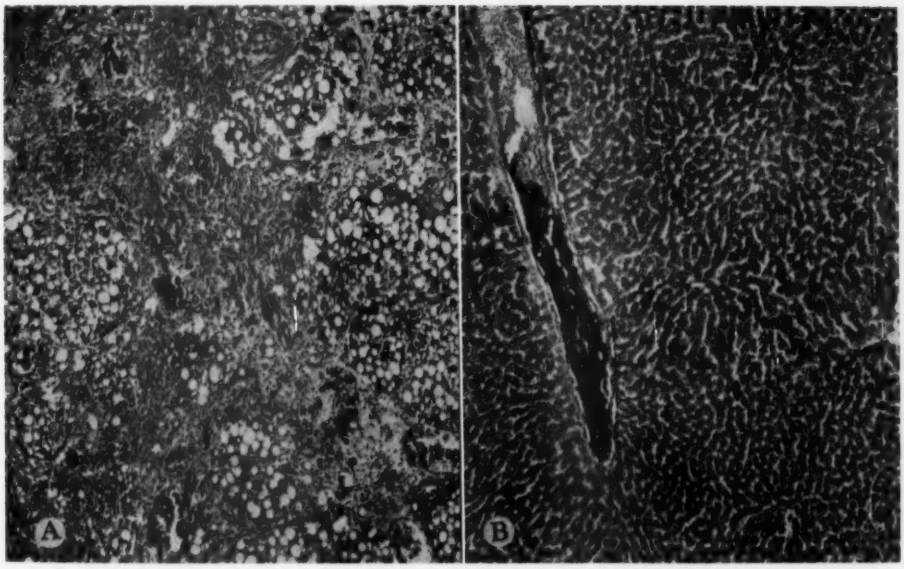


Fig. 2 (Rat 12).—*A*, liver biopsy tissue after six months on the cirrhosis-producing diet. There is disruption of the normal architecture with increase in connective tissue, which is infiltrated with lymphocytes. The liver cells are vacuolated, indicating fatty changes. This lesion was graded as 4+. Hematoxylin-eosin stain; reduced to 77% of mag. × 100.

B, liver at autopsy after 10 months on the commercial Chow diet, showing return to normal architecture. The excessive fibrous tissue and cellular infiltration have disappeared. The liver cords have an orderly arrangement and do not contain visible fat. This liver was graded 0. Hematoxylin-eosin stain; reduced to 77% of mag. × 100.

Fig. 3 (Rat 71).—*A*, liver at biopsy six months after the cirrhosis-producing diet was instituted. Increased connective tissue and vacuolated parenchymal cells are similar to those in Figure 1*A*. The lesion was graded 3+. Hematoxylin-eosin stain; reduced to 77% of mag. × 100.

B, liver at autopsy 10 months after biopsy—same regimen as that for Figure 1*B*. There is a striking decrease in the amount of connective tissue, but thin strands in excess of normal still persist. Fat vacuoles have disappeared from the liver cells, which appear normal. The lesion was graded as 1+. Hematoxylin-eosin stain; reduced to 77% of mag. × 100.

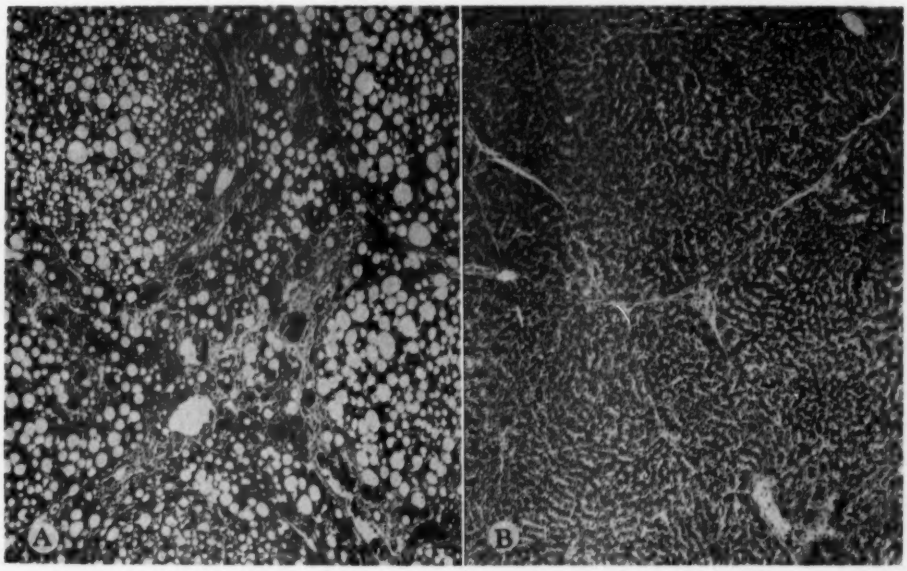


TABLE 4.—*Collagen Concentrations in Normal and Cirrhotic Rat Livers*

Groups	Collagen * Mean Values	Standard Deviation	Standard Error
1 Treated cirrhotics at 16 mo.	1.95 (range 1.25-3.50)	±0.51	±0.07
2 Controls at 16 mo.	2.01 (range 1.49-3.20)	±0.49	±0.12
3 Untreated cirrhotics at 6 mo.	2.94 (range 1.14-5.50)	±1.19	±0.27
4 Controls at 6 mo.	1.44 (range 0.93-1.96)	±0.33	±0.10

Significance of Differences Between Means of Pairs

	Difference	Stand. Error	t	Probability
Cirrhotic rats at 6 mo. *vs.* controls at 6 mo.	1.50	±0.39	3.87	<0.01
Treated cirrhotic rats at 16 mo. *vs.* controls at 16 mo.	0.06	±0.14	0.43	0.7
Cirrhotic rats at 6 mo. *vs.* treated cirrhotic rats at 16 mo.	0.99	±0.11	8.68	<0.001

* Milligrams per 100 mg. fat-free, dry liver tissue.

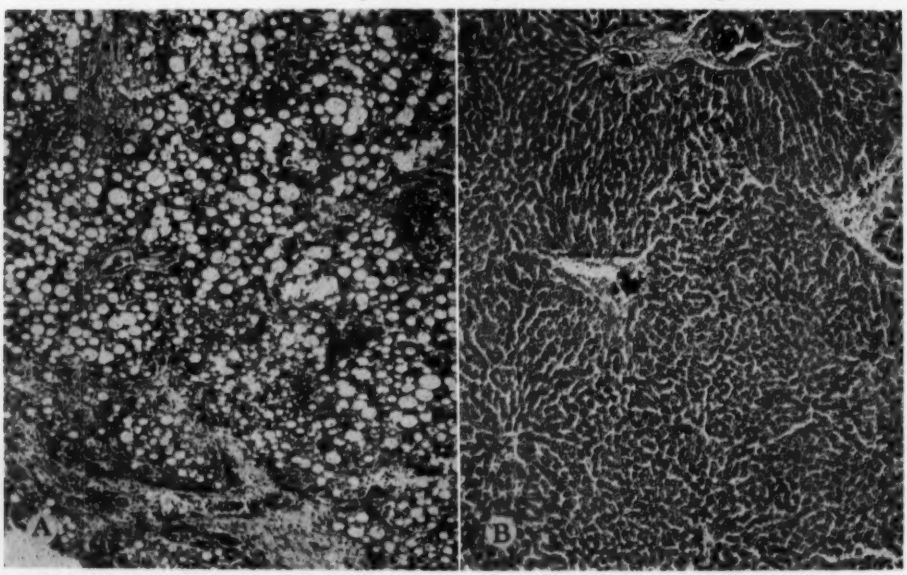
Determinations of liver collagen concentration were carried out at 6 and at 16 months (Table 4). At the end of the fore period of six months, the livers of normal rats contained 1.44 mg. per 100 mg. of dry, fat-free liver weights, whereas the livers of cirrhotic rats had roughly twice this concentration, 2.94 mg. per 100 mg. (Table 4, Groups 3 and 4).

At the end of 16 months comparison was made of the treated cirrhotic rats and their corresponding normal controls (Table 4, Groups 1 and 2). The collagen values for these two groups were statistically the same, namely, 2.01 and 1.95 mg. per 100 mg. Apparently, the treated cirrhotic animals had experienced a return to normal values of their liver collagen.

It is noteworthy that the liver collagen for normal controls averaged 1.44 mg. per 100 mg. at 6 months and 2.01 mg. per 100 mg. at 16 months. An increase in the concentration of collagen in blood vessels [15] and liver [16] coincident with aging has been observed by others. It is possible that the increase noted here is related to denser supporting tissue about the vascular structures.

Fig. 4 (Rat 10).—*A*, liver biopsy after six months on the 4% casein diet. The liver lesion was graded 4+. Hematoxylin-eosin stain; reduced to 77% of mag. × 100.

B, liver 10 months after biopsy—animal on commercial Chow diet. Note slightly increased fibrosis about the portal triads, particularly about the largest one in the field. The residual lesion was considered 1+. Hematoxylin-eosin stain; reduced to 77% of mag. × 100.



Comment

The present studies indicate that in rats with experimental cirrhosis produced by diets deficient in protein and lipotropic factors the change to a nutritious diet, adequate in balanced protein, brings about disappearance of fat, of lymphocytic cellular infiltration, and of connective-tissue overgrowth. There is restoration of normal-appearing liver cells and structure. These changes imply that there is a mechanism for the removal of connective tissue, since the values for liver collagen of the treated animals were virtually the same as for the normal controls.

Cameron and Karunaratne,[12] and Sellers, Lucas, and Best [13] have observed the histological disappearance of connective tissue from the livers of rats recovering from carbon tetrachloride poisoning. Morrione,[14] also, has demonstrated a progressive decrease of collagen, histologically and chemically, in livers of rats recovering from carbon tetrachloride cirrhosis. These experiments indicate that the deposition of collagen is reversible. It may be argued that carbon tetrachloride cirrhosis in the rat does not have its counterpart in human cirrhosis.

It may also be questioned whether cirrhosis of the liver in the rat produced by means of a protein-deficient diet has its human counterpart. The history of patients with Laennec's cirrhosis characteristically reveals an inadequate diet, particularly lacking in meat and dairy foods. The excessive intake of alcohol by these patients increases the dietary imbalance by providing calories without nutrients. The dietary background is not unlike that of the experimental animal. The histological changes also show convincing similarities. Insofar as human disease can be reproduced in the rat, the analogy between experimental dietary cirrhosis and human Laennec's cirrhosis seems close.

There is little evidence thus far in the medical literature that histological repair in the human is as complete as that described in these animals. There is a dearth of experience in this respect. Few cases have had sequential biopsies over a period of years. Moreover, there are few subjects who would provide a good study, since the majority revert to their former alcoholism. If the normal life span of the rat is roughly three years, then the 10-month period of therapy corresponds to about 20 or 25 years of man's life. This analogy may not be valid, but it serves to emphasize that dietary treatment has occupied a large share of the rat's life and that corresponding data in man probably should involve many years of treatment.

Summary and Conclusions

Male rats of the Sprague-Dawley strain were placed on a protein-deficient (4% casein) diet for six months. At this time, biopsy tissue from the liver was obtained. About 70% of the rats exhibited diffuse fatty cirrhosis. A sample group of animals were killed and their livers analyzed chemically for collagen. The remaining rats with cirrhosis of the liver were placed on a nutritious Chow diet for 10 months, at which time they were killed, their livers examined microscopically, and the collagen concentration of their livers quantitated.

Comparison of findings at the time of biopsy and at autopsy showed that in animals fed the Chow diet there had been disappearance of fatty changes and lymphocytic infiltration and of connective-tissue overgrowth, with a return of normal-appearing parenchyma. In rats with moderate degrees of cirrhosis at six months, the recovery appeared to be complete at the time of autopsy. In those with severe liver cirrhosis there often remained a few fine strands of connective tissue at autopsy.

Values for liver collagen, measured as hydroxyproline, showed corresponding changes. Untreated animals with cirrhosis had liver collagen values of about twice the normal. In contrast, the liver collagen concentration of treated animals was that of the normal controls.

In experimental dietary cirrhosis of the liver, the increased connective tissue disappears with appropriate treatment. The mechanism by which this takes place has not been determined.

Research Service Columbia University Division, Goldwater Memorial Hospital, Welfare Island (17).

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Regeneration in Fatty Liver and Cirrhosis

Autoradiographic Study Using Tritiated Thymidine

RICHARD A. MacDONALD, M.D.; RUDI SCHMID, M.D., and G. KENNETH MALLORY, M.D., Boston

Cirrhosis is usually defined histologically in terms of the three components, regeneration, necrosis, and fibrosis.[1] The factors responsible for regeneration, however, and whether it is present in the developmental stages of cirrhosis or occurs only as part of a repair process after cirrhosis is well established have not been clarified. In the present work it was found that in the cirrhosis that follows fatty infiltration of the liver, new-cell formation or regeneration precedes the onset of cirrhosis. Two experiments were carried out; the first was a study of the degree of new-cell formation in each stage of fatty liver progressing to cirrhosis in rats which were fed a choline-deficient diet. The formation of new cells was assessed by histologic criteria, which included counting mitoses, and by quantitating the number of tritium-labeled hepatic-cell nuclei in autoradiographs prepared after administration of tritiated thymidine.[2] In the second experiment an attempt was made to determine further that in fatty liver an increased uptake of tritiated thymidine by hepatic cells was due to new-cell formation. The autoradiographic technique utilizing tritiated thymidine was employed because the short beta emission of tritium (hydrogen-3) permits preparation of detailed cellular autoradiographs, and thymidine is considered to be a specific precursor only for the synthesis of deoxyribonucleic acid (DNA). DNA is confined to the nucleus of the cell and is believed to be formed only when a cell prepares for subsequent division.[2,3] An injection of tritiated thymidine, therefore, followed by biopsy of tissues or killing the animal, and preparation of autoradiographs permits detection of nuclei that were synthesizing DNA in preparation for division at the time of the injection.

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Mallory Institute of Pathology, Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital; Departments of Pathology and Medicine, Harvard Medical School, and Department of Pathology, Boston University School of Medicine.

Materials and Methods

EXPERIMENT 1.—Ninety-three male rats of the Sprague-Dawley strain* were used in the first experiment. In this, 51 animals were fed a choline-deficient diet, and 42 were litter-mate controls, 37 of which were fed standard Purina Laboratory Chow and 5 were pair-fed the deficient diet with 2% choline added. There was no difference in results in the two control groups, so that they are hereafter considered together. Findings in 51 animals, 30 on the deficient diet and 21 controls, are summarized in the Table. Animals not included in the Table were used as follows: Six were fed the choline-deficient diet and, after reaching a stage of 3+ fatty infiltration of the liver, were fed a 5% choline supplement for two weeks and then killed. Five litter mates serving as normal controls were fed a standard diet. Thirty-one additional animals on the choline-deficient diet were used to check the weight of the liver at a stage when 3+ fat was present.

The choline-deficient diet was similar to that used previously[4]; peanut meal was fed in place of arachin and 30% fat in place of 10% fat. Animals were housed in individual cages in an air-conditioned room and were fed the diets ad libitum, beginning when they were approximately 30 days old, and weighing approximately 100 gm. After maintenance on the diet for various periods of time, from seven days to 14 months, one or

*Charles River Breeding Labs., Cambridge, Mass.

more rats on the deficient diet, together with one or more control animals, was given concurrently a single intraperitoneal injection of tritiated thymidine,† 1.0μc/gm. body weight. Four hours later, the rats were killed by decapitation; histologic sections and autoradiographs were prepared, using a stripping film method.[8] All sections were cut at 6μ. The amount of fat and the degree of fibrosis in the liver were determined in histologic sections, using an arbitrary scale of 1+ to 4+. In four livers fat was measured chemically[9] to determine the approximate correlation between the amount of fat and the histologic grading. Two livers with 3+ fatty infiltration of the liver contained 23% and 27% fat by dry weight; two normal rat livers contained 4% and 6% fat.

In histologic preparations and in autoradiographs an eyepiece grid marker was used to quantitate hepatic cells. In autoradiographs, the number of "labeled" hepatic-cell nuclei (those bearing the tritium label), present in 100 consecutive × 400 fields was counted. In accompanying sections stained with hematoxylin and eosin, the total number of hepatic-cell nuclei present per × 400 field was determined by averaging the results obtained from counting five such fields. From these determinations, the number of labeled hepatic-cell nuclei in autoradiographs was expressed per 100,000 hepatic-cell nuclei. In determining mitotic activity, the number of mitoses present in 50 consecutive × 400 fields was counted in hematoxylin-eosin sections, and values were expressed per 100,000 hepatic-cell nuclei. At this magnification, cells in late prophase, metaphase, telophase, and anaphase were counted. If no mitotic figures were observed in 200 consecutive × 400 fields, the mitotic count was designated as zero. Cells in fibrous bands, whether labeled or unlabeled, were not included in cell counts.

EXPERIMENT 2.—In the second experiment 23 rats were fed a choline-deficient diet until a markedly fatty liver, graded as 3+ to 4+, was present. A single intraperitoneal injection of tritiated thymidine, 1.0μc. per gram, was then administered, and two days later a small biopsy specimen of the liver, approximately 0.1 gm., was obtained surgically. Ten days later the rats were killed, and a second portion of the liver was obtained. The two samples of liver were handled identically; sections were cut together; autoradiographic film was placed over sections at the same time, and the details of developing and staining the films were identical. The number of mitoses and of tritium-labeled hepatic nuclei was quantitated, as in Experiment 1. In addition, in autoradiographs grain counts were made over individual labeled hepatic nuclei in both specimens. In each autoradiograph 20 consecutive labeled hepatic nuclei were photographed at × 800 magnification, using oil immersion with an objective of N.A. 1.25. A 2.0 mm. micrometer was photographed at the same magnification in order to measure the diameter and thereby to calculate the area of each labeled nucleus. In photomicrographs the number of exposed granules in the stripping film overlying each labeled nucleus was counted. The results were expressed in terms of the number of granules per square micron of nucleus, to allow for variation in size of individual nuclei.

† Schwarz Laboratories, Mount Vernon, N.Y. Specific activity 0.360 curie/mM.; beta energy of tritium 0.0179 mev.

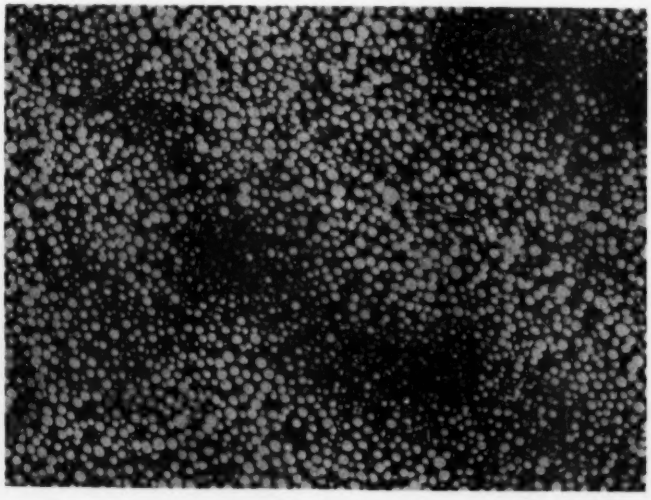

Fig. 1.—Liver with 3+ fatty infiltration. At this stage, as the degree of fatty infiltration increases, portal areas also become filled with fat, and an increase in uptake of tritiated thymidine by hepatic nuclei throughout the liver becomes apparent in autoradiographs, preceding the onset of cirrhosis. Hematoxylin-eosin stain; reduced to 68% of mag. × 100.

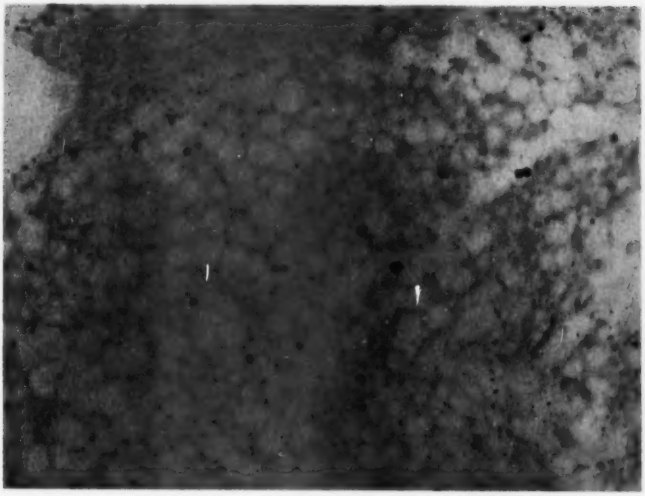

Fig. 2.—Autoradiograph of liver with 3+ fatty infiltration, showing several tritium-labeled hepatic nuclei, distributed uniformly throughout hepatic parenchyma. Hematoxylin-eosin stain; reduced to 68% of mag. × 100.

Results

EXPERIMENT 1.—When fed the choline-deficient diet, animals developed fatty liver after varying times, depending upon their consumption of the diet; rats that ate greater quantities of the diet mixture developed fatty liver and cirrhosis more quickly. The number of tritium-labeled hepatic cells in autoradiographs and the number of mitoses in histologic sections appeared to be related to the degree of hepatic fat and was independent of the length of time that animals were fed the diet. Figure 1 illustrates the appearance of a 3+ fatty infiltration of the liver. When livers reached this degree of fatty infiltration, an increase in labeling in autoradiographs became apparent (Fig. 2), and an increase was found in the number of mitoses in histologic sections. Quantitative data for tritium-labeled hepatic nuclei and mitoses are given in the Table. In rats with a slight degree of hepatic fat, graded 1+ to 2+, the number of labeled cells and mitoses was not significantly different from their controls. In rats with 3+ or more fat, however, an increase in the number of labeled cells over that in control animals was significant beyond the 0.01 level by the *t*-test, and for mitoses, the difference was significant at the 0.05 level. When a choline supplement was fed for two weeks to animals that had been on the deficient diet, and that had been presumed to have had 3+ fatty liver, the number of labeled nuclei and

Uptake of Tritiated Thymidine and Mitotic Activity in Livers of Rats Fed a Choline-Deficient Diet

Rats Fed a Choline-Deficient Diet					Litter Mates Fed a Control Diet				
Fat *	No. of Rats	Mean Weight, Gm.	Tritium Labeled Hepatic Nuclei †	Hepatic Mitoses ‡	Fat *	No. of Rats	Mean Weight, Gm.	Tritium Labeled Hepatic Nuclei †	Hepatic Mitoses ‡
1+	8	145	440.6	3.1	0	6	209	507.7	21
2+	7	123	482.0	3.9	0	5	194	412.8	26.6
3+-4+	15 §	265	1,649.2	84.5	0	10	364	119.3	23.7

* Graded histologically as 1+ to 4+.
† Expressed per 100,000 hepatic nuclei in autoradiographs.
‡ Expressed per 100,000 hepatic nuclei in histologic sections.
§ Five rats of this group had cirrhosis in addition to fatty liver.

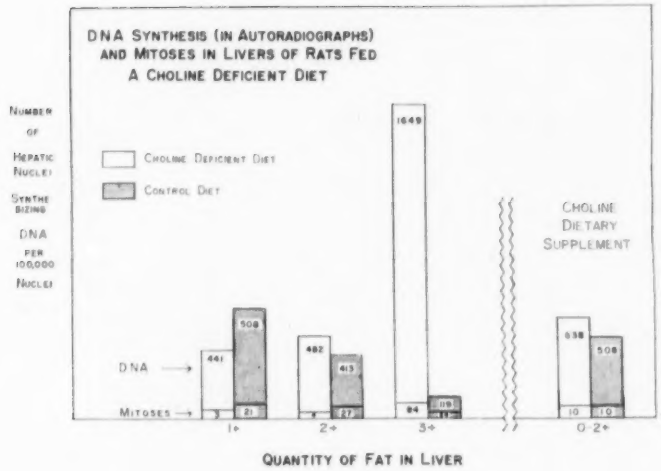

Figure 3

of mitoses was found to be comparable to controls fed a standard diet (Fig. 3).

Labeled hepatic-cell nuclei were randomly distributed throughout the liver, and were not localized to regenerating foci or nodules (Fig. 2). Nuclei of cells comprising fatty cysts frequently showed labeling, as did polyploid hepatic nuclei. When fibrosis was developing, elongated nuclei of cells considered to be fibroblasts were labeled.[7] Kupffer cells usually showed labeling, both in livers of rats on a deficient diet and those fed a standard diet; labeling of bile-duct epithelium was less frequently observed. Focal necrosis was occasionally present in livers with 3+ or more fat and was noted as a focus of polymorphonuclear leukocytes and cellular disintegration affecting one to three hepatic cells, but necrosis was not a prominent feature at any stage. When 3+ or more fat was present, livers were approximately twice normal weight, apparently owing to both fat content and an increase in the number of hepatic cells. In rats with 3+ fatty liver the average liver weight was 22.3 ± 5.2 gm. compared with 12.4 ± 2.3 gm. in control rats. Further, the number of hepatic-cell nuclei per $\times$ 400

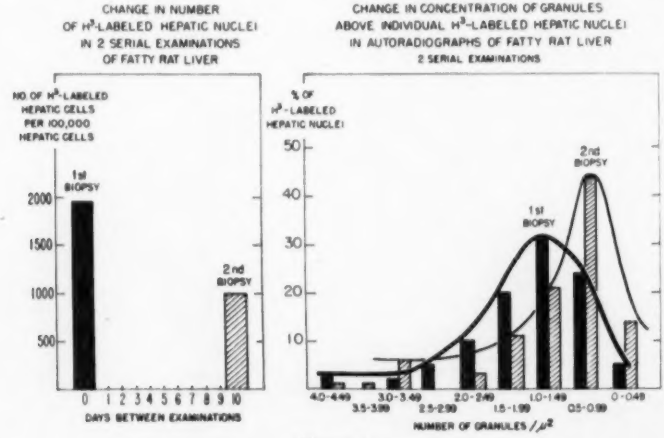

Figure 4

field in fatty livers was not statistically different from control livers. In livers with 3+ or more fat, the average number was 170.7±36.9, while in normal livers the average was 189.1±44.0.

EXPERIMENT 2.—When a specimen of liver was obtained 10 days after an initial biopsy in rats with markedly fatty liver (3+ to 4+ fat), the number of tritium-labeled hepatic nuclei in autoradiographs was approximately 50% of the number present in the initial biopsy (Fig. 4). This suggested that labeled cells had either undergone necrosis or had further divided, thus diluting their labeled DNA until it was no longer detectable in autoradiographs. The latter explanation was considered more likely because of the finding that in the specimens taken 12 days after the injection of tritiated thymidine, the number of granules per square micron of hepatic nucleus was less than in the first specimen (Fig. 2). However, some degree of liver-cell death could not be ruled out by this method.

Comment

The findings in these experiments indicate that in fatty livers in rats, increased formation of hepatic cells is present before cirrhosis is detectable. Further, they suggest that the degree rather than the mere presence of fat is of importance. It was not determined whether the fat itself is responsible for the increase in hepatic-cell formation or whether it is merely an associated finding. In human studies, the importance of fat in causing one type of cirrhosis has been a matter of controversy.[8] For example, it has been noted that a slight degree of fat may be present in the liver without being followed by cirrhosis. Similarly, diabetics frequently have a slightly increased amount of fat in the liver, but do not have more cirrhosis than control populations.[9] If fat plays a causal role in hepatic cirrhosis, it would appear that a slight degree of fat accumulation does not exert the same deleterious effect that is caused by a larger quantity.

It is considered significant that new hepatic-cell formation or regeneration in fatty liver was uniformly distributed throughout the liver; there was no localization in periportal or centrilobular regions. "Nodular regeneration" was not evident and was therefore not considered to be of importance during the developmental stages of the cirrhotic process, although in animals with well-advanced cirrhosis nodular regeneration became apparent.

Summary and Conclusions

Formation of new cells was studied at each stage in the development of fatty liver and cirrhosis in rats fed a choline-deficient diet for periods of 7 days to 14 months. Autoradiographs, following injection of tritiated thymidine, and quantitation of mitosis in histologic preparations were used to assess new-cell formation. A slight amount of fat in the liver was not associated with an increase in hepatic-cell formation. When the livers became markedly fatty, however, formation of new hepatic parenchymal cells was increased, and this preceded the onset of cirrhosis.

Technical assistance was given by Helen Cadegan, Jane Donelan, and Lorraine Martin.

Mallory Institute of Pathology, Boston City Hospital (18).

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Cardiac Hypertrophy After Coronary Artery Ligation in Rats

TOM D. NORMAN, M.D., and CARL R. COERS, M.D., Little Rock, Ark.

There has been considerable speculation as to whether occlusive coronary artery disease causes myocardial hypertrophy. Evidence in man favoring this concept has been presented by Connolly and Littmann [1] and by Davis and Blumgart.[2] However, the problem has been difficult to study in human beings because of associated variables, especially hypertension. Thus it was decided to investigate the effect of coronary ligation upon heart weight in the rat.

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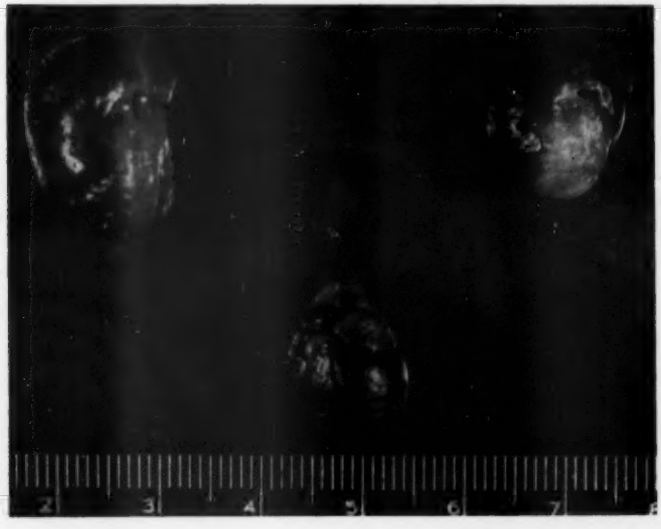
Departments of Pathology, University of Mississippi and University of Arkansas Medical Centers, Jackson, Miss. and Little Rock, Ark.

Present addresses: Department of Pathology, University of Arkansas Medical Center, Little Rock (Dr. Norman); University of Texas Medical Branch Hospitals, Galveston, Texas (Dr. Coers).

Materials and Methods

Positive pressure artificial respiration was instituted after tracheal intubation of male Holtzman rats anesthetized with intraperitoneal pentobarbital, and the hearts were exposed by a left parasternal incision. The anterior descending coronary artery was ligated just below its origin (Figure) in 23 rats, and a sham operation, with placement of an epicardial suture adjacent to the artery at that level, was performed on 23 control animals. An additional 26 animals did not survive operation and were not studied further. The rats had free access to Purina Laboratory Chow and water at all times.

Eleven coronary-ligated and 11 control rats were killed by exsanguination 6 weeks after operation, and 12 coronary-ligated and 12 control animals were killed 12 weeks afterward. Each heart was excised by cutting across the blood vessels at their junction with the heart, and opened, washed, and blotted dry before being weighed on a Voland balance. The atria were excised from the ventricles and the right ventricle from the septum; the left ventricle, with attached septum, and the right ventricle were then weighed separately. Portions



Coronary-ligated hearts (note sutures) of animals killed 12 weeks after operation, above, have large healed infarcts and are larger than the heart from a sham-operation control without an infarct, below. This control heart was paired with the heart above and to the left, and weighed only slightly more than one-half (1.1 gm.) as much as the coronary-ligated heart (1.9 gm.).

TABLE 1.—*Mean Body and Heart Weights of Coronary-Ligated* and Sham-Operation Rats*

Group	No. Rats	Initial Body Wt., Gm.	Terminal Body Wt., Gm.	Heart Wt., Gm.	Heart-Body Wt. Ratio (10^{-4})	Left Vent., Gm.	Right Vent., Gm.	Lt.-Rt. Vent. Ratio (10^{-1})
Six weeks								
Ligation	8	177	304	1.121	37.2	0.665	0.231	30.3
Sham operation	11	176	322	0.975	30.3	0.681	0.156	45.5
Twelve weeks								
Ligation	12	159	385	1.388	36.2	0.897	0.275	36.1
Sham operation	12	158	391	1.176	30.2	0.840	0.203	41.3

* Only those coronary-ligated rats which developed gross infarcts are included.

of the apex of the left ventricle, the septum, and the psoas muscle were placed in a drying oven at a temperature of 90 to 100 C for three or more days, until a constant weight could be recorded. The spleen, liver, kidneys, and adrenals from each rat were also weighed and, with lungs, thyroid, left ventricle, and septum, were prepared for histologic studies after fixation in 10% formalin. Mean initial body weights were similar, and since only a small difference was present terminally (Table 1), especially in the animals killed 12 weeks after operation, a *t*-test was done to show significance of the difference of the means of various organ weights and ratios and muscle water content.

Results

Large, healed infarcts were present in 8 of 11 coronary-ligated rats killed at 6 weeks and in all 12 coronary-ligated rats killed at 12 weeks. Grossly discernible hypertrophy was present in many of the hearts with infarcts, both in the 6-week and in the 12-week groups (Figure).

There was marked thinning of the infarcted areas, where muscle was replaced with fibrous connective tissue, in rats killed 12 weeks after operation. Similar, but less fibrous, infarcts were present in hearts of coronary-ligated rats killed six weeks after operation. No significant histologic abnormalities were noted in the various other organs studied, including kidneys and thyroids. Mild chronic pneumonitis was present in treated and control animals but was of similar degree in the two groups.

Table 1 indicates the number of rats in each group, initial and terminal body weights, heart weights, and heart-body and left- right-ventricle ratios. P was 0.05 for the difference in mean heart weights at 6 weeks and 0.02 for the difference at 12 weeks. This was considered statistically significant, especially since body weights of the control rats were slightly greater than those of the coronary-ligated animals. That P was 0.005 at 6 and 12 weeks, when the heart-body weight ratios were considered, supports this conclusion; however, the same slight difference in terminal weights that made the P values of more significance in the first comparison makes this one slightly less significant. There was a significant difference also between the left- right-ventricle ratios, at 6 weeks, but not at 12 weeks, indicating a disproportionate increase in the weight of the right ventricle in the earlier period. Left-ventricle weights of coronary-ligated animals were less than those of control rats at 6 weeks after operation but were

TABLE 2.—*Mean Water Content of Tissues in Coronary-Ligated and Sham-Operation Rats*

Group	No. Rats	Myocardial Water—Apex, %	Myocardial Water—Septum, %	Psoas Muscle Water, %
Six weeks				
Ligation	8	77.9		77.3
Sham operation	11	74.9		77.8
Twelve weeks				
Ligation	12	76.9	78.0	74.9
Sham operation	12	75.4	76.1	76.0

TABLE 3.—*Mean Organ Weights of Coronary-Ligated and Sham-Operation Rats*

Group	No. Rats	Liver Wt., Gm.	Spleen Wt., Gm.	Kidney Wt., Gm.	Adrenal Wt., Gm.
Six weeks					
Ligation	8	11.8	0.9	2.2	0.042
Sham operation	11	12.4	0.8	2.4	0.045
Twelve weeks					
Ligation	12	15.9	1.2	3.0	0.060
Sham operation	12	15.3	1.1	3.1	0.066

greater than those of control animals at 12 weeks (P value of difference in body-left ventricle ratio, 0.005). This difference was regarded as statistically significant.

Table 2 shows the water contents of the myocardium and psoas muscle; no significant difference was noted between coronary-ligated and control animals. Myocardial water studies were made to determine whether change in water content could account for change in heart weight, while psoas muscle water studies were to determine whether congestive heart failure occurred in these animals.

Table 3 shows the mean weights of various other organs; no statistically significant difference was found between the animals with coronary ligation and those with sham operation.

Comment

An increase in heart size and weight after ligation of the anterior descending coronary artery was demonstrated in these experiments. As myocardial water was not increased to a significant degree, and as increase in heart weight not accounted for as water is generally acknowledged as hypertrophy, it is believed that the increased weight represented cardiac hypertrophy.

Friedberg and Sohval[3] recognize as causes of cardiac hypertrophy those factors "which (1) either increase the resistance to outflow from the heart, (2) increase the inflow to the heart, or (3) produce severe myocardial weakness." Hearts or rats whose coronary arteries were ligated would clearly fall into the last of these categories. This is supported by evidence that myocardial infarcts are accompanied by a decrease in cardiac output.[4]

It would be expected that such damage would produce left ventricular hypertrophy, which occurred in the 12-week rats, but not in the 6-week animals (Table 1). The occurrence of right ventricular hypertrophy (actual, as well as relative) was unexpected. Since there is good evidence that congestive heart failure may cause some degree of cardiac hypertrophy,[1,2] such a mechanism may have been responsible for the right ventricular hypertrophy. The lack of an increase in water content of the psoas muscle and the absence of histologic evidence of chronic passive congestion in the lungs do not completely rule out this possibility. It is considered extremely unlikely that the presence of a slight chronic pneumonitis, of similar degrees in both control and treated animals, played a part in the right ventricular hypertrophy.

The present investigation, as well as studies demonstrating heart hypertrophy in chronic anemia,[5,6] appeared to be opposed to the thesis that an adequate blood supply is necessary for hypertrophy to develop. On the other hand, hypertrophy did not occur in the left ventricle, the ischemic region of the hearts, in animals killed at six weeks. Since hypertrophy did occur in the infarcted ventricle of animals killed 12 weeks after the operation, the possibility exists that reestablishment of the blood supply to that ventricle was necessary for hypertrophy to develop. The argument in the case of anemic heart hypertrophy stands no better because circulatory adjustments in chronic anemia appear to insure against the development of myocardial ischemia or

anoxia.[7,8] It still is not clear, then, whether hypertrophy will develop in the ischemic heart. No doubt part of the confusion is due to so little being known of the basic mechanisms of hypertrophy.

Although the conditions of the experiments being reported are not strictly analogous to the problem in man, where there is diffuse occlusive coronary disease, it is believed that this study gives additional support to the thesis that heart hypertrophy may result from coronary artery disease.

Summary

The left descending coronary arteries of rats were ligated and the hearts of these animals were studied for evidence of hypertrophy 6 and 12 weeks after the operation by comparison with controls undergoing sham operation. There was an increase in weight of ligated hearts at both time intervals. Hypertrophy of the left ventricle occurred only in rats studied 12 weeks after operation, but the right ventricle was increased in weight at both 6- and 12-week intervals after the ligation. It is concluded that under certain conditions occlusion of the coronary arteries results in myocardial hypertrophy.

Department of Pathology, University of Arkansas Medical Center.

The technical assistance of Judith Forbes, Mary Ann Isard, and Suzanne Haggard is gratefully acknowledged.

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Demonstration of Esophageal Varices by Simple Technique

BERNHARD CHOMET, M.D.; LEONARD M. HART, and FRANK J. REINDL, A.B., Chicago

Introduction

Demonstration of varices of the esophagus at the autopsy table is a difficult task. Collapse of the varicosities,[1] postmortem digestion, and opacity of the mucosa make it almost impossible to visualize the distended veins. Reliance merely on the autopsy studies will result in seriously erroneous impressions of the incidence of esophageal varices.[2] Injection or inflation of the veins will yield a surprising picture of the extent of the dilated veins.[1] Since injection of the esophageal veins is not likely to be utilized routinely, a simpler method for demonstration of esophageal varices is certainly desirable.

After fixation in formaldehyde, the esophageal wall appears grossly to be composed of two layers—the mucosa and the muscularis. Both are held together by loose connective tissue, which constitutes the submucosa. It is relatively easy to separate the mucosa from the muscularis. If one works close to the muscularis when stripping the mucosal layer, most of the submucosa, and thus most of the submucous veins, remain attached to the mucosa. The submucous venous plexus is poorly supported by loose connective tissue.[3]

After separation, the mucosa and the attached submucosa are taken through some changes of alcohol and into benzene solution for clearing. Then the specimen is photographed while it is still in benzene. This clearing method demonstrates quite dramatically the submucous venous plexus of the esophagus.

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From the Clinical Laboratory and Medical Illustration Service, Veterans Administration West Side Hospital and the Chicago Medical School.

Chief, Laboratory Service, Veterans Administration West Side Hospital, and Assistant Professor of Pathology, Chicago Medical School (Dr. Chomet). Assistant Chief (Mr. Hart) and Chief (Mr. Reindl) Medical Illustration Service, Veterans Administration West Side Hospital.

Technique

1. Place esophagus, without folding, into 10% formaldehyde solution.

2. After 24 hours' fixation, strip the mucosal layer from the muscularis very carefully so as not to tear the specimen.

3. Completely submerge mucosal layer in absolute alcohol. Three changes of alcohol are necessary. Allow one-half hour between changes, and keep specimen flat at all times.

4. Dry specimen, then completely submerge in a tray of benzene. Keep tray covered. Clearing time varies; average time is 15 minutes.

5. Dry specimen and transfer to opal glass tray (dental instrument tray) containing insoluble ruler and filled with benzene. Specimen must be completely submerged during photography. The tray is covered with a sheet of glass.

6. Arrange lights. Three are necessary: One light is placed under tray for transillumination (be sure to mask all other areas around tray with black cardboard); the other two are placed at equal distances, pointing toward top of specimen.

7. Photograph and return specimen to fixing solution.

Results

A definite pattern of the submucous veins is noted in the cleared esophagus of noncirrhotic patients. Despite minor variants, certain basic features can be seen. The veins, two to three in number, traverse the esophagus longitudinally as though originating at the cardia, and continue parallel to each other with relatively minor cross communications (Fig. 1). In some cases the vessels appear to be connected

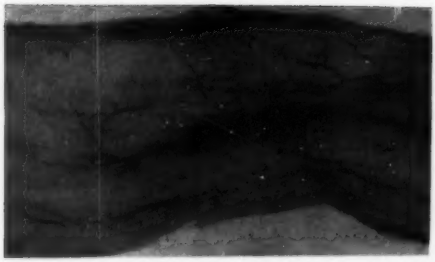


Fig. 1.—Normal esophagus showing parallel veins with only minor cross communications. × 1½.

Fig. 2.—Esophagus in cirrhosis showing dense network of capillary vessels. × 2½.

with another set of veins that is seen in the proximal esophagus. In other cases the veins extend throughout the entire length of the esophagus. Variations of the pattern are conditioned by the amount of clotted blood left in the veins after death, the extent of venous collapse, the state of preservation, the care in preparation of the specimen, and most important, the underlying disease.

In cirrhosis a radical departure from the basic pattern becomes apparent. The esophagus shows an abundant tapestry of probably newly formed small vessels. They form a dense, almost uniform, network. The impression gained from the photograph is that of hemorrhage. Only higher magnification makes it possible to observe the dense mosaic of newly formed small blood vessels (Fig. 2). There are cases in which the abundance of small vessels obscures the presence of varicose veins. In other cases, distended, tortuous large veins dominate the picture, while the network of newly formed small blood vessels is less conspicuous (Fig. 3). The varicose veins are mainly situated in the distal end of the esophagus.

Occasionally, we noted varices in patients without evidence of cirrhosis. In chronic cardiac disease the esophagus may show development of a venous pattern that ranges from a normal picture to one approaching the pattern seen in cirrhosis (Fig. 4). The postmortem collapse of veins and occasional inadequate clearing may at times give a false gross impression. In these cases the microscopic sections help in the visualization of an abnormal venous pattern.

Fig. 3.—Esophagus in cirrhosis showing tortuous varicose veins. × 1½.

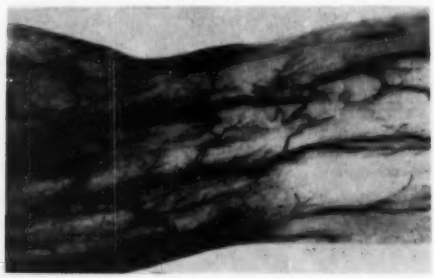
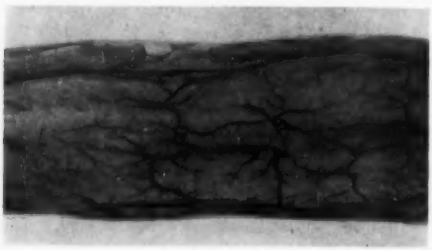


Fig. 4.—Esophagus in chronic cardiac disease showing increased vascularization. × 1½.



Summary

A new method is described for demonstration of the submucous venous pattern of the esophagus. This technique has been helpful in evaluating the vascularization of the esophagus in patients with cirrhosis. The method depends upon the loose attachment of the mucosa to the muscularis of the esophagus. Stripping of the mucosa

with the attached submucosa, and clearing of the specimen in benzene, will result in a realistic demonstration of the submucous veins.

Laboratory Service, Veterans Administration West Side Hospital, 820 S. Damen Ave. (12).

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The Size of Muscle Fibers in Infants and Children

D. H. BOWDEN, M.B., Ch.B., and R. A. GOYER, M.D., St. Louis

The estimation of the size of muscle fibers is a valuable procedure in the diagnosis of the disorders which affect skeletal muscle.

The variation of fiber size, which is a feature of normal muscle, may be greatly accentuated in muscular and neuromuscular diseases. The fibers may be arranged in distinctive patterns, so that the diseases which affect primarily the muscle cell may be distinguished from those which result from lesions of the nervous system. An intimate admixture of fibers of greatly varying size is characteristic of the myopathies, whereas the motor neuron diseases exhibit distinct groupings of small and large muscle fibers.

Such patterns are readily identified, but there are occasions when a knowledge of the normal size of various muscle fibers at different ages is useful in the evaluation of a muscle biopsy.

The muscle fibers of normal adults may show as much as a fivefold variation of diameter within the same muscle, and in the fetus the difference may be even greater (Halban [1]). Similarly, the average size of fibers within one muscle differs from the mean diameter of the fibers of another muscle.

The variations among different muscles in the same person are probably related to function; a strong muscle is usually a big muscle, and the total muscle mass is directly related to the size of the individual fibers of the muscle.

In the embryo, the fibers of all the muscles are of similar size, and they appear to grow in fairly uniform manner until birth (Halban [1]). The only observations concerning the sizes of muscle fibers in children are those recorded by Wohlfart [2] (1937). The results of the present investigation supplement those figures.

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Fellow of the National Foundation (Dr. Goyer).

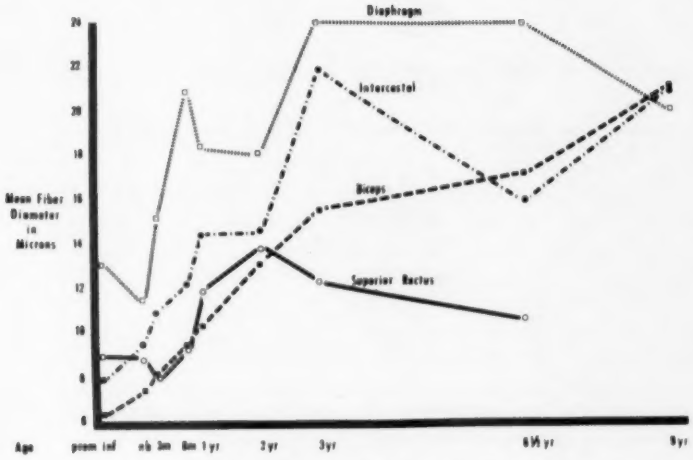
From the Departments of Pathology, St. Louis University School of Medicine, and Cardinal Glennon Memorial Hospital for Children.

Methods

The measurements were made on muscles obtained at autopsy from nine infants and children, varying in age from birth to 9 years. All of the patients died following illnesses of short duration and apparently not related to the neuromuscular systems. No other selection was attempted. Pieces of muscle measuring 2×0.5 sq. cm. were excised with a sharp knife or razor blade and placed on a narrow strip of filter paper with the fibers parallel with the longer side of the strip. A few minutes

Mean Diameters, with Standard Deviations, of Skeletal Muscle Fibers

Muscle	Age of Patient								
	4 Lb. Premature	Newborn	3 Mo.	8 Mo.	1 Yr.	2 Yr.	3 Yr.	6½ Yr.	9 Yr.
Diaphragm	13.2(1.7)	11.5(1.6)	15.2(3.7)	20.7(3.5)	18.2(3.4)	17.9(3.9)	24.0(3.7)	23.8(4.8)	19.6(5.1)
Intercostal	6.7(1.5)	8.4(1.8)	10.9(2.5)	12.0(2.5)	14.5(2.5)	14.5(2.5)	21.8(3.6)	15.7(3.0)	20.9(3.4)
Deltoid	6.3(1.3)	6.8(1.6)	9.8(3.0)	8.2(3.0)	10.3(6.4)	10.3(6.4)	19.8(6.3)	21.4(3.8)	20.7(3.1)
Biceps	6.1(1.3)	7.3(1.8)	8.4(1.9)	9.5(2.2)	10.2(2.9)	13.3(3.7)	15.4(4.1)	16.9(2.4)	20.8(3.9)
Gastrocnemius	7.1(2.2)	6.2(1.9)	9.1(1.7)	8.0(1.4)	16.0(2.8)	18.8(3.7)	15.3(2.0)	21.1(2.7)	29.1(4.6)
Vastus lateralis	10.3(1.8)	6.2(1.9)	10.0(1.6)	..	11.6(1.8)	15.0(4.5)	14.4(2.6)	20.4(3.4)	26.3(5.6)
Gluteus maximus	7.1(2.4)	6.3(2.2)	8.7(2.6)	..	10.8(2.5)	15.1(1.8)	16.8(4.3)	24.0(3.2)	23.7(2.6)
Superior rectus	8.7(3.3)	8.6(2.0)	8.1(1.9)	9.6(2.5)	11.8(2.9)	13.8(3.1)	12.3(3.8)	10.8(1.6)	..



Variation of muscle fiber size with age.

were allowed for adherence before immersion in Zenker's fixative. After fixation, the end of the tissue was cut off and the two pieces embedded to provide longitudinal and transverse sections of the muscle fibers. Sections were cut at 5μ and stained with hematoxylin and eosin. In each muscle examined, the diameters of 100 fibers were measured, using a calibrated microscopic ocular scale. The fibers were examined at × 97 (oil immersion) magnification, and only fibers seen in exact cross section were measured. No attempt was made to determine the relative numbers of the Wohlfart a and b fibers; instead, 100 fibers of various sizes were measured and the mean diameter and standard deviation calculated.

Results

The mean diameters of the fibers of different muscles and the standard deviations are shown in the Table, and representative muscles are illustrated in the Figure.

Comment

The results suggest that the size of skeletal muscle fibers is directly related to function. In the fetus the fibers of the various muscles closely approximate each other in size, but by the time of birth the growth of the diaphragm has outstripped that of all the other striated muscles. Respiration, the only significant activity of skeletal muscle in the neonatal period, is predominantly diaphragmatic in nature, and the fibers of the diaphragm are almost twice the diameter of the intercostal and limb muscles.

As voluntary movements increase during the first year, so the locomotor muscles enlarge, until they approximate the size of the respiratory muscles. Thereafter, these muscles are composed of fibers of similar size, whereas the delicate extrinsic muscles of the eye are very little larger than they are at birth.

The changes described closely parallel the work loads of the muscles and support the opinion of Halban [1] that muscles increase in size and power by growth of the individual fibers.

Summary

The mean cross-sectional diameters of several skeletal muscles have been determined in nine infants and children. The results suggest that the size of muscle fibers is directly related to function.

Department of Pathology, Cardinal Glennon Memorial Hospital, 1465 S. Grand Blvd. (4).

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Pathology of Experimental Virus Hepatitis in Mice

BORIS RUEBNER, M.D., and JAMES L. BRAMHALL, M.B., Ch.B., Halifax, N.S., Canada

No laboratory animal has yet been found to be susceptible to the human hepatitis virus.[1] All experimental work on virus hepatitis has, therefore, had to employ one of the hepatotropic animal viruses. The mouse hepatitis viruses (MHV) isolated by Nelson [2] and by Dick, Niven, and Gledhill [3] are among those which have been used in experimental work.[4,5] The discoverers of the mouse hepatitis viruses studied the histopathology of these infections after intraperitoneal injection of the virus.[2,3] Gledhill and associates [3] appear to be the only workers who have compared the mortality after intraperitoneal injection with the effect of subcutaneous and intravenous injection. They used only a few small groups of mice and did not study the histopathology of the animals injected subcutaneously or intravenously. The present investigation was undertaken as a preliminary to studying the effect of various dietary factors on experimental virus hepatitis.[6,7] It amplifies previous work on the histopathology of this infection after intraperitoneal injection [2,3] and on the differences in mortality after infection by different routes.[8] It also describes the histological findings after intravenous and subcutaneous infection which do not appear to have been investigated before. An attempt is made to explain the differences in mortality after injection by different routes in terms of the histopathology of this infection.

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Present address (Dr. Ruebner): Pathology Department, The Johns Hopkins Hospital, Baltimore 5.

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From the Department of Pathology, Dalhousie University Faculty of Medicine, and the Institute of Pathology, Province of Nova Scotia.

Material and Methods

Mice.—Female mice, aged 21 days, of the Swiss Webster strain, supplied by Budd Mountain Farm, Chester, N.J., were used. Repeated blood examinations have shown these mice to be free from Eperythrozoon coccoides which enhances the severity of the infection. The animals were fed an unrestricted diet of Purina Chow.

Virus.—The MHV3 strain of mouse hepatitis virus was used in the form of a 10% suspension of mouse liver obtained from infected animals. Mortality due to this virus decreases with age. After intraperitoneal injection of 0.1 ml. of the virus suspension the mortality in this strain of mice is approximately 60% at the age of 21 days.

Experimental Methods.—Ninety mice were divided into three groups of thirty each. One group was infected by intraperitoneal injection; the other groups received injections into the subcutaneous tissue on the back and into the tail vein, respectively. The same virus pool was used for the different routes of inoculation. After injection, the cages were inspected daily, and the number of dead mice in each group was noted. The livers from all dead animals were examined histologically.

The histology of the lesions due to the virus and their developments were investigated in a further experiment, in which three groups of 30 mice each were injected by the intraperitoneal, subcutaneous, and intravenous routes. One mouse from each group was killed daily and subjected to a full histological examination. The blocks were fixed in Carnoy's fluid. Paraffin sections were stained with hematoxylin and eosin, and many by Van Gieson's method, the periodic acid-Schiff reaction with and without diastase digestion, von Kóssa's stain for calcium, and Lendrum's method for reticulin.[9] Frozen sections for fat were stained with Sudan IV. The distribution of the earliest lesions was studied by serial sections.

Results

Mortality.—The activity of the animals injected intraperitoneally or intravenously was decreased from the third day after infection. On the fourth day the first deaths occurred in these two groups. The mortality was always highest in the group injected

Number of Mice Dying Each Day After Injection of Hepatitis Virus

Mice Injected	Days												Deaths, Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Intraperitoneally	0	0	0	5	9	1	2	0	1	0	0	0	18
Intravenously	0	0	0	3	6	4	2	1	0	0	0	0	16
Subcutaneously	0	0	0	0	1	0	1	4	2	0	0	0	8

intraperitoneally. After intravenous injection the mortality was slightly lower. After subcutaneous injection, however, the number of deaths was always less than half that in the other two groups. Moreover, the deaths in the group injected subcutaneously occurred two to three days later than in the groups injected by the intraperitoneal or intravenous route.

The Table shows the number of mice dying each day after infection in one experiment. There was clearly no difference in mortality between the group injected intraperitoneally and the group injected intravenously. The mortality after subcutaneous injection, however, was significantly less than after intraperitoneal injection ($\chi^2=5.5$; $P=0.02$) and the greatest number of deaths occurred on the eighth day as compared with the fifth day. No deaths occurred after the ninth day, and a few days later the mice were clinically normal.

Gross Observations.—The abnormalities at postmortem examination were confined to the abdominal cavity. Three days after intraperitoneal or intravenous infection a few pinhead-sized, yellow areas could be seen on the surface of the liver. These rapidly multiplied and enlarged during the next two or three days until the liver surface had a yellowish mottled appearance. The lesions were diffusely distributed through all lobes. After subcutaneous infection the changes generally developed later and were less conspicuous. If the animals recovered, the gross appearance of the liver returned to normal after two to three weeks. A few mice showed a moderate amount of clear yellow ascitic fluid. This was seen particularly during recovery from the hepatitis in the second week after infection.

Histology.—Sections taken 24 hours after intraperitoneal injection showed a few groups of acute inflammatory cells on the

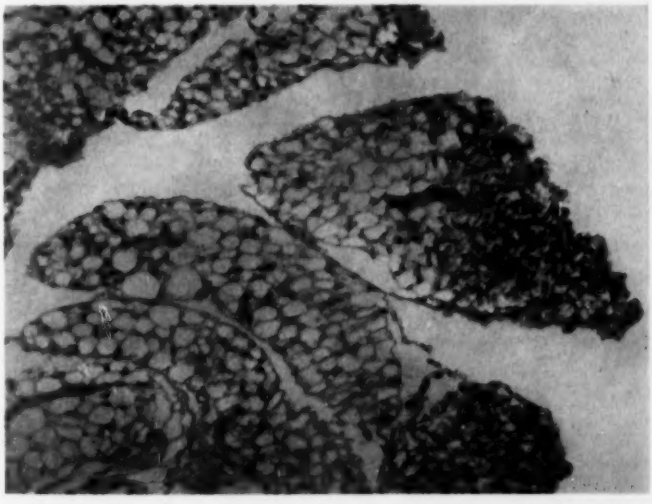

Fig. 1.—Acute inflammatory reaction on the surface of omental fat. Mouse killed 24 hours after intraperitoneal injection of MHV3. Hematoxylin and eosin stain; reduced to 85% of mag. × 50.

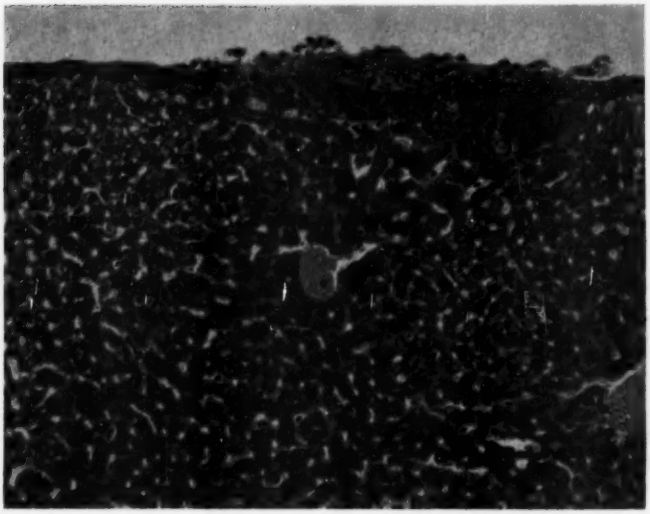

Fig. 2.—Necrotic area with acute inflammatory reaction at the liver surface. Animal killed two days after intraperitoneal injection of MHV3. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

surface of the omental fat (Fig. 1). After two days the first hepatic lesions appeared. These consisted of small foci of necrosis at the surface of the liver (Fig. 2). Both the capsule and the underlying parenchyma were affected. In the damaged cells nuclear and cytoplasmic changes took place almost simultaneously. First there was increased prominence of the nucleoli and distention of the nuclear membrane. The cytoplasm, which in control animals was free from stainable fat, showed mild fatty vacuolation. There was also a decrease in the normally well-defined basophilic granules of Berg, which are believed to be ribonucleic acid aggregates.[10] The cytoplasmic glycogen was markedly diminished. Karyorrhexis, karyolysis, and nuclear pyknosis soon followed, and the cytoplasm showed irregular, fine basophilic stippling, followed by eosinophilic necrosis. Polymorphonuclear-cell infiltration was seen in all but the smallest

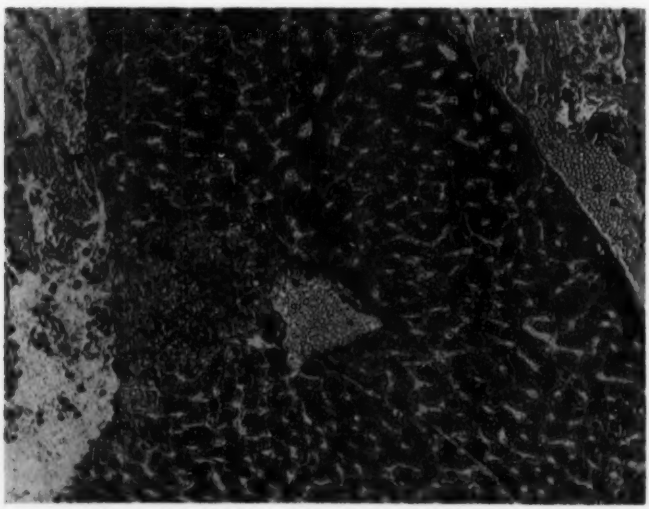

Fig. 3.—Liver from mouse killed three days after intraperitoneal injection of MHV3. One of the necrotic areas has extended into a centrilobular vein. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

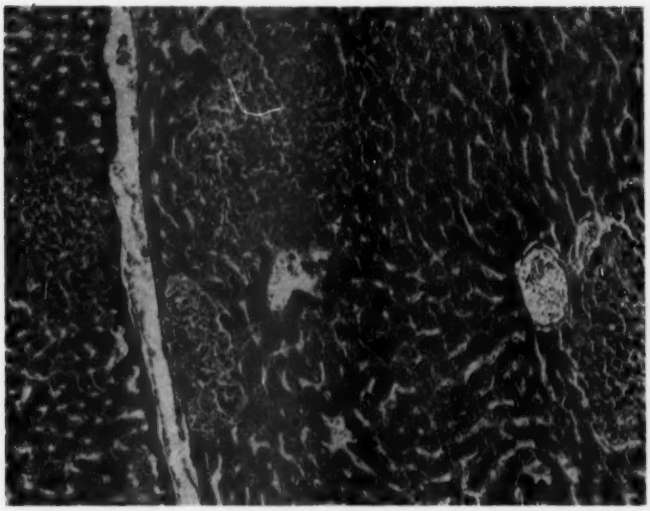

Fig. 4.—Another area from the same specimen as that in Figure 3. Foci of necrosis are seen both on the surface of the liver and in the substance of the liver in relation to centrilobular veins. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

necrotic foci. On the third day the areas of necrosis had enlarged and penetrated deeper into the parenchyma. Many had extended into the centrilobular veins (Fig. 3). Satellite foci could be seen deep in the substance of the liver in relation to branches of the hepatic veins (Fig. 4). Amorphous material which was faintly positive to the PAS reaction even after diastase digestion could often be seen in the necrotic foci. After four days the greater part of the liver was necrotic (Fig. 5), and the reticulin network was severely damaged (Fig. 6). If the animals died, there were only a few surviving liver cells, most of which surrounded portal triads. These cells usually were greatly distended with stainable fat (Fig. 7). If the animals survived, marked histiocytic infiltration developed, and intense mitotic activity was seen after the sixth day. Bile stasis was not observed. After 10 to 14 days there was almost complete regeneration of the liver, with only slight distortion of the lobular pattern and mild

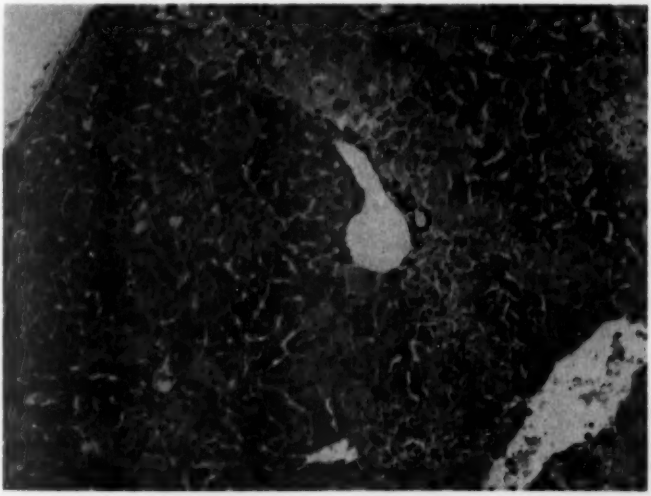

Fig. 5.—Liver from mouse killed four days after intraperitoneal injection of MHV3. The greater part of the organ is necrotic. Hematoxylin and eosin stain; reduced to 85% of mag. × 50.

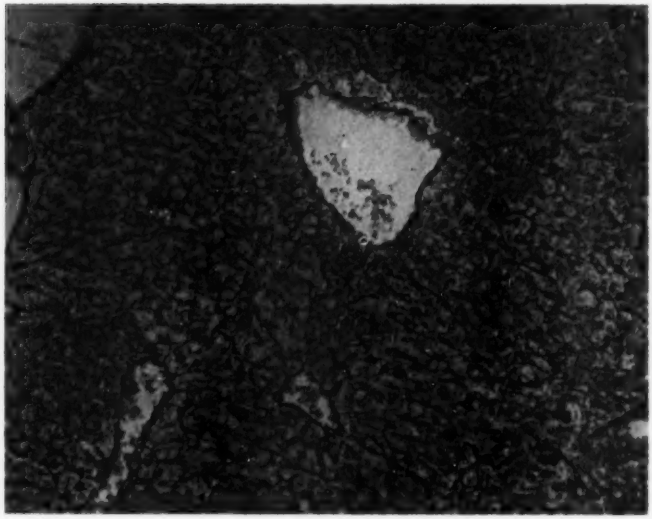

Fig. 6.—Same specimen as that in Figure 5, showing a damaged reticulin network in two areas adjacent to centrilobular veins. Reticulin stain; reduced to 85% of mag. × 150.

infiltration of the portal areas with foci of chronic inflammatory cells. Apart from some increase in capsular fibrous tissue no parenchymal fibrosis was seen. The reticulin network appeared to be actually diminished. Nodular hyperplasia of the liver cells also was not encountered. Foci of necrosis similar to those in the liver were noted during histological examination of the lymph nodes (Fig. 8) and adipose tissue of the abdomen and in the spleen (Fig. 9).

After intravenous injection the liver lesions differed in distribution from those occurring after peritoneal injection. Instead of beginning on the surface of the liver and spreading into the parenchyma, the earliest foci were already widely scattered through the liver (Fig. 10). These lesions were first noted after 48 hours and showed no preference for any particular zone in the liver lobule. During the next two days these foci increased in size and coalesced until,

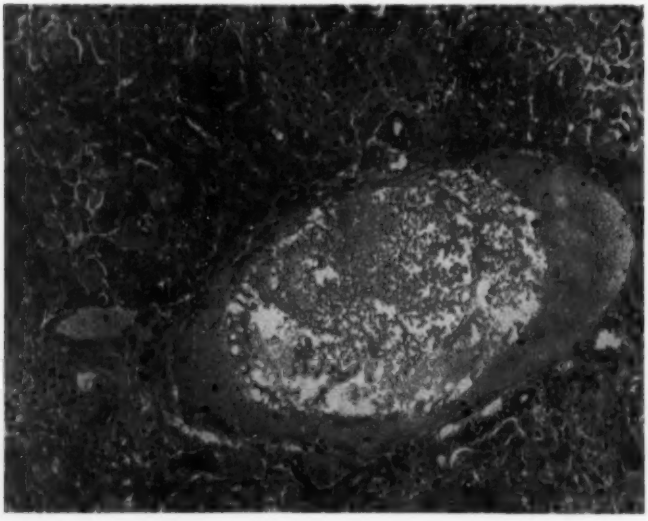

Fig. 7.—Liver from mouse which died four days after intraperitoneal injection of MHV3. Only a few surviving liver cells are seen, most of which surround portal triads. They are vacuolated, owing to fatty change. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

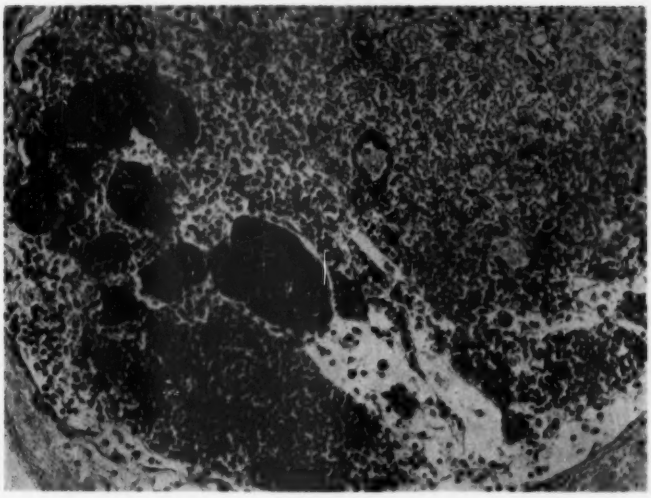

Fig. 8.—Abdominal lymph node with an area of necrosis three days after intraperitoneal injection of MHV3. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

on the fourth day, the appearance of the liver was indistinguishable from that four days after intraperitoneal injection. The lesions in the lymph nodes, adipose tissue, and spleen were also similar to those produced by intraperitoneal injection. Rapid and complete regeneration of the liver occurred in the surviving animals.

Following subcutaneous injection, the first histological evidence of damage occurred after 24 hours in the subcutaneous fat at the injection site. At that time a few acute inflammatory cells appeared in the adipose tissue. After 48 hours, the inflammatory reaction was greatly intensified (Fig. 11). Control mice receiving subcutaneous injections of heat-inactivated virus showed minimal inflammatory changes only. After subcutaneous injection of MHV3 liver lesions were not seen until the fourth day. The appearance of the liver at this stage was similar to that two days after intra-

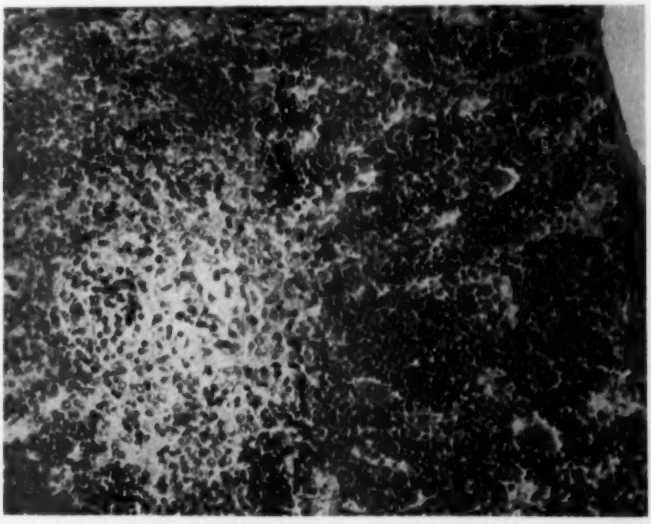

Fig. 9.—Spleen with a focus of necrosis four days after injection of MHV3. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

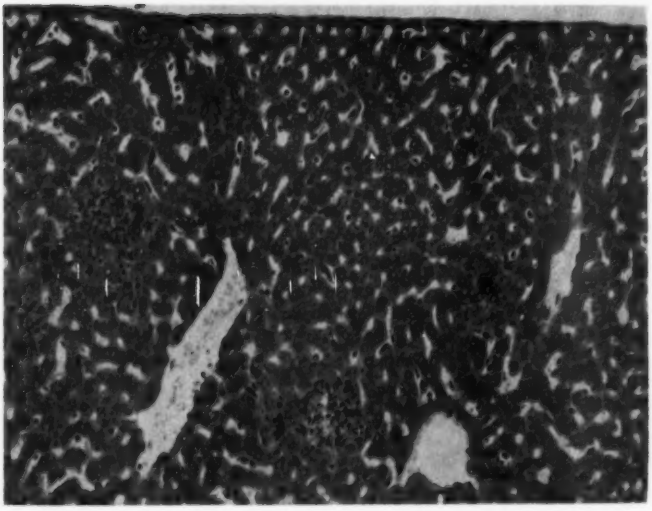

Fig. 10.—Liver from mouse killed two days after intravenous injection of MHV3. Small foci of necrosis are scattered throughout the liver without preference for any particular zone of the liver lobule. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

venous injection. The subsequent development of these lesions resembled that following intravenous infection. The lesions did, however, continue to lag about 48 hours behind those produced by the intravenous route and tended to be less extensive. The extrahepatic lesions resembled closely those produced by intraperitoneal and intravenous infection. In this group, also, healing of the liver took place without any evidence of cirrhosis.

Comment

The histological appearances noted after intraperitoneal injection were similar to those previously described after infection by the MHV group.[2,3,8] The early mesothelial giant-cell reaction noted by Dick, Niven, and Gledhill[3] was inconspicuous in our experiments. That liver involvement begins on the surface and has a tendency to spread along the centrilobular veins was suggested by Dick, Niven, and Gledhill.[3,8]

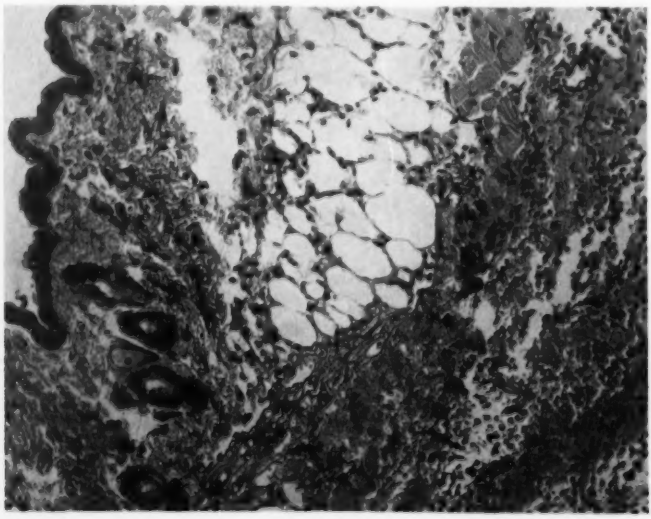

Fig. 11.—Acute inflammatory reaction at the injection site two days after subcutaneous infection with MHV3. Hematoxylin and eosin stain; reduced to 85% of mag. × 150.

The diminution in glycogen and the increase in stainable fat have not been mentioned in previous reports. The damage to the reticulin network and the deposition of PAS-positive material in the necrotic foci also do not seem to have been observed before. In spite of the damage to the reticulin network, fibrosis[8] and cirrhosis,[2] which had been noted in previous work on this infection, were conspicuously absent in those of our mice which recovered from the infection. The faintly PAS-positive material seen in the necrotic foci probably represented serum.[11]

The infectivity of the virus after intravenous injection was comparable to that injected by the intraperitoneal route. This was previously observed by Gledhill and associates,[8] who did not, however, examine this group of animals histologically. Although mortality after intravenous injection was comparable to that occurring after intraperitoneal injection, there was a marked difference in the distribution of the early hepatic lesions. After intravenous injection many foci of necrosis appeared simultaneously in the parenchyma instead of spreading from the surface of the liver, as after intraperitoneal injection.

After subcutaneous injection the mortality was less than half that produced by the intraperitoneal or intravenous route. This is in keeping with the findings of Gledhill, Dick, and Niven,[8] who did not report any histological studies of animals injected by this route. We found that after subcutaneous injection the earliest lesions occurred at the injection site. Liver lesions resembling those occurring after intravenous injection were not seen until 48 hours later than after injection by the intravenous route. We believe that the delayed spread of the virus to the liver may allow the animals to develop an immunity sufficient to lower the mortality significantly and to increase the period of incubation.

The lesions produced by the mouse hepatitis group of viruses, while resembling closely those produced by dietary necrosis in rats,[10,12] differ in some respects from those of human viral hepatitis.[13] In the mice the lesions are focal, whereas human hepatitis affects the liver diffusely. Fatty change and damage to the reticulin framework are frequent in mice but are rarely seen in human cases. It appears that in mice severe damage to the reticulin framework, with almost complete loss of its argentophilia, is no bar to regeneration of the liver in a normal pattern. Bile stasis, which is common in man, was not observed in mice. This may have been due to the acuteness of the infection, but it is possible that bile thrombi were dissolved by the chloroform in Carnoy's fixative. Eosinophilic necrosis, periportal inflammation, and liver-cell regeneration are, however, seen in both infections, and the histological appearance of the liver in fatal infections with MHV3 resembled that of acute yellow atrophy, in which there is almost complete hepatic necrosis apart from a few surviving liver cells in the periphery of the lobules.

In spite of these differences from human hepatitis, infection by the mouse hepatitis group of viruses represents one of the few available experimental models for the study of virus hepatitis. The experiments described above suggest that the difference in incubation period between human infectious hepatitis and serum jaundice may be due to the different routes of infection and not to any immunologic differences between the viruses.

Summary

The mortality and histopathology produced by intraperitoneal injection of a mouse hepatitis virus (MHV3) were compared with the effect of injection by the subcutaneous and intravenous routes. After subcutaneous infection the mortality was less than half that produced by intraperitoneal or intravenous infection, and the greatest number of deaths occurred three days later than in the other two groups.

After intraperitoneal injection the first lesions occurred in the abdominal fat and

on the surface of the liver. The liver lesions extended into the centrilobular veins, and satellite foci developed deep in the substance of the liver in relation to branches of the hepatic veins. After intravenous injection the earliest foci were already widely scattered through the substance of the liver and showed no preference for any particular zone in the liver lobule. Following subcutaneous injection the first lesions occurred in the fat at the injection site. The liver lesions resembled those produced by intravenous injection but lagged approximately 48 hours behind them. In the surviving animals, liver regeneration took place in 10 to 14 days. Although the reticulin framework appeared to be damaged, regeneration occurred in a normal pattern.

The histopathology of human infectious hepatitis is compared with that of the experimental infection produced by MHV3. It is suggested that the difference in incubation period between human infectious hepatitis and serum jaundice may be due to the different routes of infection and not to any immunologic differences between the viruses.

We wish to thank Mr. C. S. Brindle for taking the photographs and Miss Ora Ashley for technical assistance.

Department of Pathology, The Johns Hopkins Hospital, Baltimore 5.

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Histologic Alterations in Muscles of Guinea Pigs During Chronic Hypoxia

ENRIQUE VALDIVIA, M.D.; MARGARET WATSON, M.Sc., and C. M. S. DASS, Ph.D., Madison, Wis.

Introduction

Natives of the Peruvian highlands are able to work and tolerate physical activities in a manner similar to that of persons at sea level. Hurtado and his collaborators [1] have demonstrated that persons living at high altitudes are even more efficient when exercising on a treadmill than are subjects at sea level. They have also found that mountaineers accomplished their tasks with a lower production of lactate. Respiratory, hematological, and cardiac changes are described in subjects living at high altitudes. These changes improve the transport of oxygen to the tissues, but the partial pressure of oxygen on the arterial side of the capillaries is lower than that from the same site in persons at sea level. An increase in the total capillary bed has been observed in the skeletal muscles of guinea pigs native to the Peruvian mountains.[2] This increased capillarity facilitates the diffusion of oxygen to the tissues. Mercker and Schneider [3] have described dilatation of cerebral capillaries in rats exposed to simulated high altitude. They indicate that such capillary dilatation improved the diffusion of oxygen.

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Department of Pathology, University of Wisconsin Medical School.

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The general process of adaptation to chronic hypoxia ultimately depends upon intracellular alterations that will enable the tissues to work efficiently at low oxygen tension. Mitochondria contain the system of cytochromes, flavoproteins, and pyridine nucleotides, which are also known as intracellular respiratory enzymes.[4] Oxygen utilization by the tissues requires the participation of these respiratory enzymes. It is therefore logical to anticipate that alterations will be observed in the tissues of persons adapted to chronic hypoxia.

The object of the present investigation is to study muscle sections from animals that have been exposed to experimental high altitude for prolonged periods. The number of capillaries filled with red blood cells will be utilized as an indication of the magnitude of the functional capillary bed at the time of death. Mitochondrial staining and electron microscopic studies will be used to evaluate any alterations in mitochondria and other fine structures of the muscle fibers.

Method

Adult male guinea pigs, weighing between 800 and 1,000 gm., were used in this investigation. Selection of 8 control and 12 experimental animals was made at random from litters of similar ages in our colony. Both control and experimental animals had approximately identical cage-floor areas available for free running. Standard guinea pig food, ascorbic acid (vitamin C), fresh lettuce, and water were provided freely. The animals were separated into four groups as follows: Group A consisted of eight control guinea pigs. Group B was composed of four animals that were exposed to simulated high altitude for intervals of up to three months. Group C included six guinea pigs exposed to experimental chronic hypoxia from 5 to 10 months. Animals in Groups B and C were placed in

low-pressure chambers, which were opened for feeding and cleaning every 48 hours. Group D consisted of only two guinea pigs that were exposed to simulated high altitudes for periods of 8 and 10 months, respectively; however, the exposure was continuous, with return to sea level only at the termination of the experiment. This last experiment necessitated the use of a special low-pressure chamber.[5] All three groups of experimental animals were exposed to 16,500 ft. of altitude at 20 to 25 C with humidity in the region of 45% to 90%. The humidity was increased when the animals were fed with fresh lettuce and the cups were filled with water. Control and experimental animals were killed by a quick blow to the head. Two muscles of the thigh were removed for study: the vastus lateralis and the rectus femoris. The distribution of white and red areas of these muscles has been described previously.[2]

Thin transverse slices of muscle were cut from the middle third of both muscles and fixed in Helly's fluid, and cross- and longitudinal sections 2μ in thickness were cut from paraffin-embedded material. These slides were stained with hematoxylin and eosin, and the technique described by Gurr[6] was used for mitochondria staining. Two-millimeter longitudinal strips from the middle third of both muscles, corresponding to either a white or a red portion, were fixed in Palade's fixative and processed for electron microscope photographs.

Capillary counts were made on the cross sections stained for mitochondria, the red blood cells being stained bright red with the acid fuchsin. A calibrated binocular compound microscope was used for these counts, at a magnification of $\times 630$. An expression derived for each animal corresponds to the average number of capillaries containing red blood cells surrounding individual muscle fibers. Five sections were studied, and the capillaries surrounding 500 muscle fibers were counted on each of five slides.

Capillaries and Mitochondria in Red and White Muscle in Chronic Anoxia

	Group A		Group B		Group C		Group D	
	Red	White	Red	White	Red	White	Red	White
Number of capillaries surrounding a single muscle fiber	2.4±0.032	1	3.8±0.027	1	4.1±0.032	1	4.3	1
Mitochondria	Peripheral perinuclear, interfibrillar	Few perinuclear	Like Group A slightly enlarged	Few perinuclear	Increase in number, size and density	Increase in size and density	Increase in number, size and density	Increase in size and density

Results

The vastus lateralis and rectus femoris muscles have red and white portions.[2] The alterations secondary to experimental chronic hypoxia are more distinct in the red areas (Table). Description of the white and red areas in muscles from Group A are described first in order to establish the structure found in control animals.

White Muscle.—The white portions of both muscles show in cross sections many large muscle fibers (Fig. 1), with a few smaller fibers found in the center of the bundles. The counts performed in these

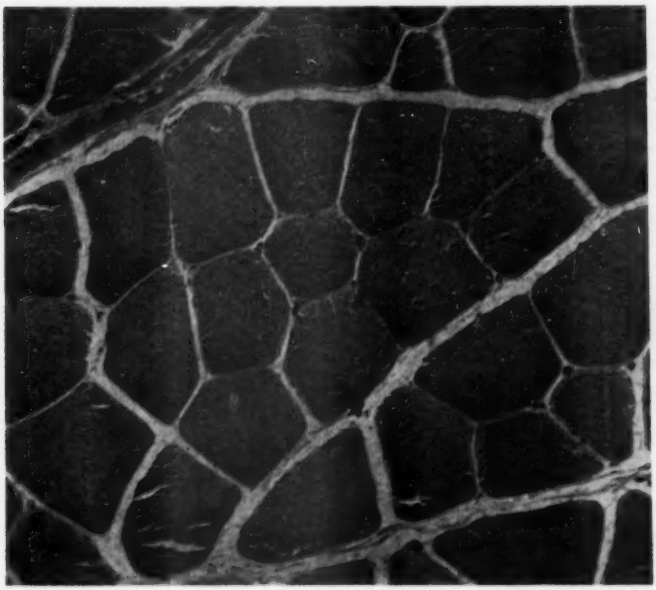

Fig. 1.—Cross section of white muscle, from vastus lateralis of the thigh. Guinea pig from Group A (control). Large muscle fibers. Few capillaries. Gurr's staining method for mitochondria; reduced to 92% of mag. × 330.

white areas average one capillary filled with red blood cells surrounding each muscle fiber. Mitochondria are found only in the periphery of the muscle fibers, and never more than four are seen in the vicinity of the nuclei. On cross sections single rows only of widely separated mitochondria are seen in the peripheral regions. Mitochondria are more abundant in the smaller fibers in the center of the bundles. The electron microscope photographs confirm these findings; i.e., mitochondria are few and sparsely

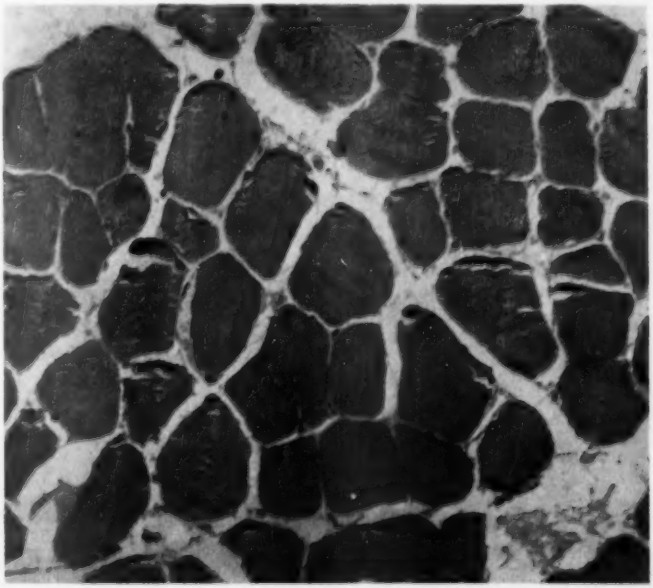

Fig. 2.—Cross section of red muscle, from vastus lateralis of the thigh. Same guinea pig (control) as in Figure 1. Smaller muscle fibers and more capillaries than in Figure 1. Gurr's staining method for mitochondria; reduced to 92% of mag. × 330.

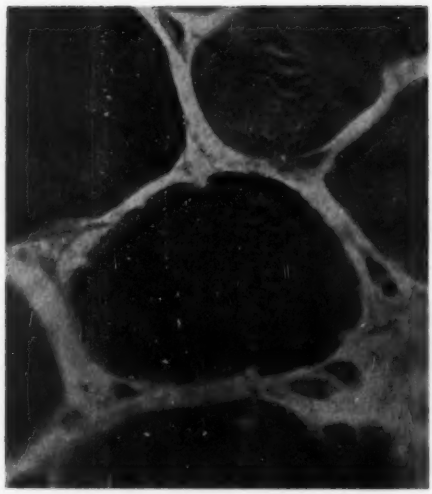

Fig. 3.—Cross section of red muscle, from vastus lateralis of the thigh. Control guinea pig from Group A. Peripheral, perinuclear, and interfibrillar mitochondria in a muscle fiber from the center of a bundle. Gurr's staining method for mitochondria; × 1,200.

distributed. The myofibrils in both the stained sections and the electron microscope photographs appear to be larger than the ones found in red muscle.

Red Muscle.—On cross sections the red muscle appears to be formed by smaller fibers than the ones described in white muscle (Fig. 2). The fibers are grouped in bundles of approximately 10 units, and they present a fairly uniform cross section. The fibers occupying the center of the bundles are smaller than the peripheral ones, and frequently the central one is the smallest (Fig. 3). The number of capillaries surrounding each red muscle fiber in control and experimental animals is represented in the Table. Mitochondria in the red muscle fibers are situated beneath the sarcolemma; they are variously shaped and form clumps around the nucleus (peripheral and perinuclear mitochondria). It is not unusual to find a capillary filled with red blood cells in the vicinity of the fiber nucleus, completing a common association, namely, mitochondria, nucleus of the muscle fiber, and capillaries (Fig. 5). Mitochondria are also seen in the central portion of the muscle fiber as slender rods situated between the myofibrils. Interfibrillar mitochondria are less numerous and less distinct in paraffin sections than are peripheral and perinuclear mitochondria.

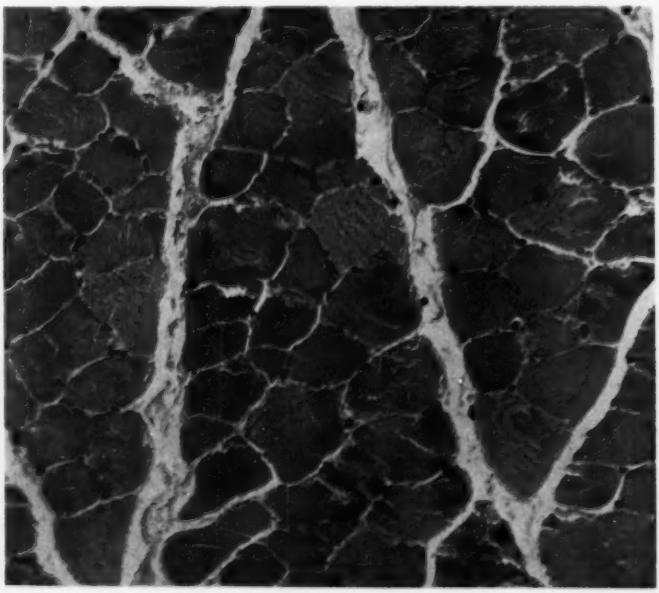

Fig. 4.—Cross section of red muscle, from vastus lateralis of the thigh. Experimental animal from Group B; six months' exposure to experimental hypoxia. Abundant capillaries. Gurr's staining method for mitochondria; reduced to 92% of mag. × 330.

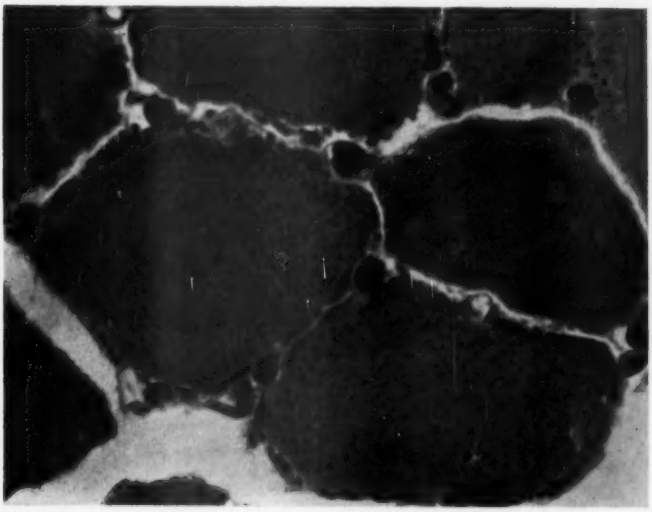

Fig. 5.—Cross section of red muscle, from vastus lateralis of the thigh. Experimental animal from Group B; six months' exposure to experimental hypoxia. Increased number of mitochondria. Numerous capillaries. Gurr's staining method for mitochondria; reduced to 92% of mag. × 1,200.

The smaller central fibers have more mitochondria than the fibers found in the periphery of the bundle. The electron microscope photographs confirm the previous description and demonstrate the fine structures more clearly.

The sections from the guinea pigs exposed to experimental chronic hypoxia for intervals of three months or less (Group B) present no significant alterations in the mitochondria population of the muscles examined. There is, however, a slight increase in the size of the mitochondria. No electron microscope studies were made on this group.

The sections, from experimental Groups C and D, of animals exposed for four or more months present a significant increase in the number of peripheral and perinuclear mitochondria; the perinuclear clumps are more distinct, and the frequent association of perinuclear mitochondria, muscle fiber nucleus, and capillary filled with red blood cells is also more evident (Fig. 5). The interfibrillar mitochondria are also increased in number, but this difference is less conspicuous. Giant mitochondria, the interfibrillar type, are very frequently found in the sections from experimental Groups C and D and are seen as rings surrounding a muscle myofibril. These differences described in the experimental Groups C and D correspond to comparable differences between muscle fibers that are known to be in the periphery and those in the center of the muscle bundles.

The electron microscopic observations confirm our previous findings. These observations are made only from fractions of muscle fibers, so that it is not known whether they are of peripheral or of central fibers. Nevertheless, mitochondria are more abundant in experimental Groups C and D (Figs. 8 and 9) than in the control groups (Fig. 7). The perinuclear clumps have more mitochondria. The mitochondria beneath the sarcolemma are also more numerous. The internal structure of mitochondria in experimental and in control animals corresponds to the general pattern described by Palade.[7] The photographs from experimental Groups C and D demonstrate that mitochondria are denser, owing to more closely packed cristae mitochondriales. The giant mitochondria found surrounding myofibrils are more numerous in the muscles of experimental animals from Groups B and C. The endoplasmic reticulum is more abundant, and it has more Palade granules in these experimental groups.

Fig. 6.—Longitudinal section of white muscle, from vastus lateralis of the thigh. Control animal. Few mitochondria, large myofibrils. *N*-nucleus; *My*, myofibril. × 20,352.

Comment

Differences between white and red muscle have been frequently described in the literature. Previous work has demonstrated a significantly greater total capillary bed in the red muscle of guinea pigs. The present study has demonstrated also that the red muscle has many more capillaries filled with red blood cells than the white areas. The counts have not been related to any absolute unit of measurement because of the uncontrollable factors of shrinking and deformity associated with preparation of the sections. These factors, however, do not detract from a relative comparison between size of the fibers and myofibrils, as well as number and distribution of mitochondria. The overall size of the muscle fibers is larger in the white areas. The myofibrils are found also to be larger in the white muscle both in sections and by electron microscopy when compared with observations on the red muscle. Marked differences are also found in the mitochondria population, the white muscle presenting relatively few peripheral and perinuclear mitochondria.

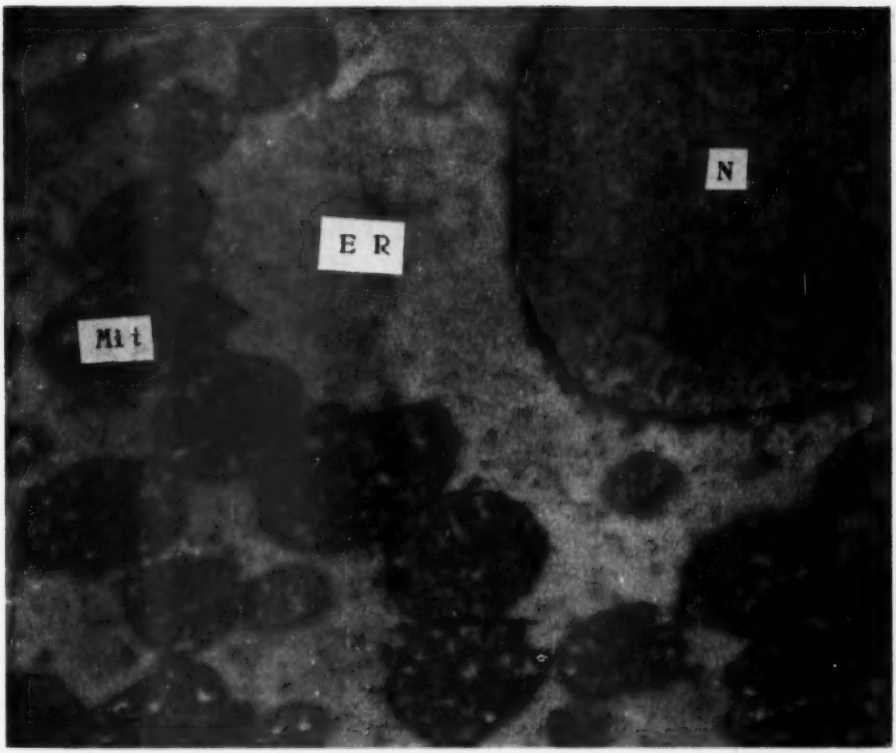

Fig. 7.—Perinuclear mitochondria, red muscle; vastus lateralis of the thigh. Control animal from Group A. *N*=nucleus; *Mit*, mitochondria; *ER*, endoplasmic reticulum. Electron microscopy; reduced to 92% of mag. × 35,488.

This investigation has also demonstrated differences between muscle fibers located at the periphery of the bundles and centrally located fibers. In both white and red muscle the more central fibers are smaller, sometimes only one very small fiber being found. The smaller central fibers contain more mitochondria than those at the periphery. These findings must be taken into consideration when sections of only parts of a single fiber are being compared, as is the case with electron microscope observations.

Differences between red and white muscle have previously been described in other small rodents. Moore and collaborators [8] found in white muscle of tibialis anterior of mice a proportion of 5 mitochondria to every 100 myofibrils. The proportion in the red fibers is 50 to 100.

Edwards, Ruska and associates [9] have also demonstrated in insects that the mitochondria of red muscle fibers are larger and more numerous than those of the less active white muscle fibers. Bennett [10] describes a model of skeletal-muscle structure representing the relationship of nuclei, myofibrils, mitochondria, and endoplasmic reticulum. Bennett's model has the structure of a highly active red muscle fiber and is not strictly comparable to our description, which corresponds to red muscle of a lower degree of activity.

The results obtained with muscle from guinea pigs exposed to experimental chronic hypoxia are highly interesting. The muscle fibers from the red areas have more mitochondria than comparable zones from the control animals. This increase in the num-

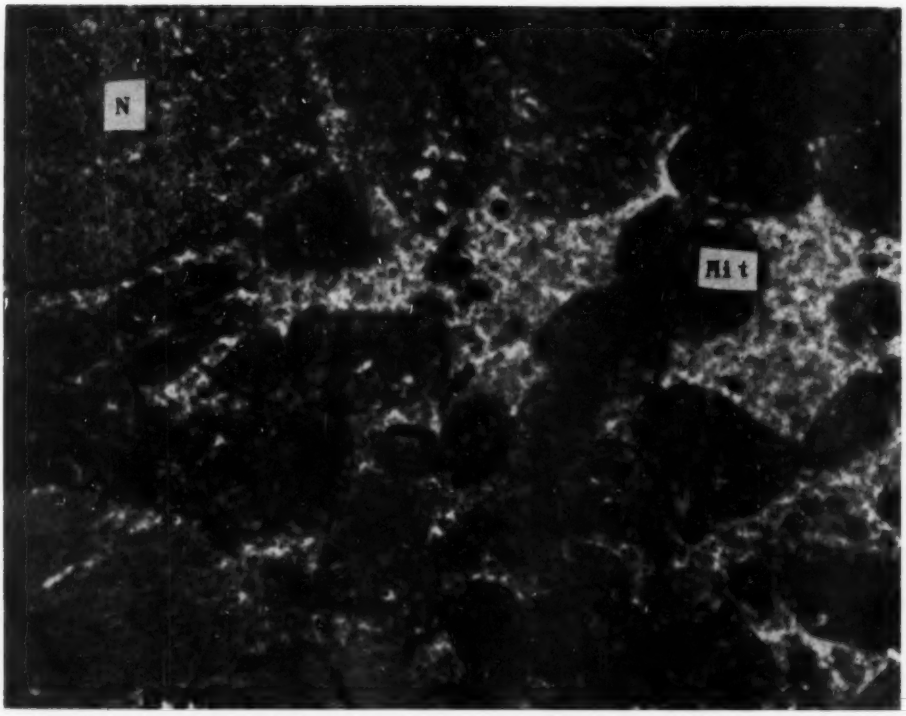

Fig. 8.—Perinuclear mitochondria, red muscle; vastus lateralis of the thigh. Experimental guinea pig from Group B; six months' exposure to experimental hypoxia. Mitochondria have densely packed cristae. Endoplasmic reticulum and Palade's granules are more distinct than in Figure 7. *N*=nucleus. Electron microscopy; reduced to 83% of mag. × 33,600.

ber of mitochondria is more distinctly observed in the peripheral and perinuclear mitochondria. The interfibrillar mitochondria are also increased in number in the red muscle of experimental Groups C and D. The interfibrillar mitochondria also are frequently found to be very large and to surround completely a myofibril. These giant mitochondria have been described in highly active red muscle fibers, but they are seldom found in our control guinea pig red muscle. The experimental Groups C and D present mitochondria that have a dense internal structure. The control animals' mitochondria have looser internal structure, with the cristae mitochondriales well separated. This difference has been described between high- and low-activity striated muscle by Edwards and Ruska.[9] Similar observations are described in other tissues by Dempsey.[11] These observations are conditioned by the relative thickness of the sections. When the sections are thick, the cristae mitochondriales are not distinct, and mitochondria appear to be dense. Our observations correspond to sections obtained by the same method. The small variations of thickness are equal in control and experimental animals and do not affect the described differences. Our results indicate that after five months of exposure to experimental chronic hypoxia the muscle fibers of guinea pigs have denser mitochondria, larger and more numerous interfibrillar mitochondria, and more numerous peripheral and perinuclear mitochondria. The changes may correspond with a better efficiency of the respiratory enzymes and with a better utilization of the available oxygen.

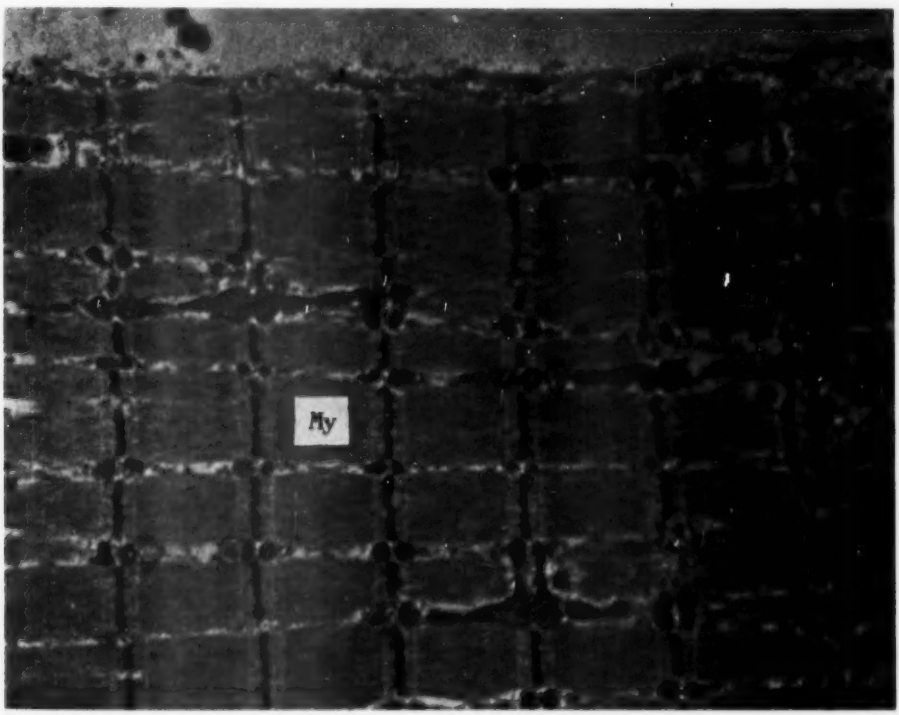

Fig. 9.—Interfibrillar mitochondria, red muscle; vastus lateralis of the thigh. Experimental animal from Group B. Large and abundant mitochondria. *My*, myofibril; Electron microscopy; reduced to 83% of mag. × 12,932.

The increase in the number of capillaries filled with red blood cells surrounding the muscle fibers favors a better interchange of gases, and it is an improvement that facilitates the diffusion of oxygen to the cell.

Summary

White and red areas of guinea pig skeletal muscle have been studied. The number of capillaries filled with red blood cells is approximately three times as great in the red areas. Mitochondria are found in greater numbers in the red areas. Mitochondria are also better demonstrated in the smaller fibers present in the central portions of the bundles in white and red muscle. The distribution of mitochondria in the muscle fibers is characteristic: Peripheral, perinuclear, and interfibrillar mitochondria are described. These findings are confirmed by electron microscopy.

The study of skeletal muscle from experimental guinea pigs subjected to simulated high altitude reveals an increase in the number of capillaries filled with red blood cells in the animals exposed for up to three months.

An increase in the capillaries and a significant increase in the mitochondria population have been observed in the red muscle from guinea pigs exposed to experimental hypoxia for intervals of four or more months. Electron microscope observations confirm the previously described findings and reveal also an increase in the Palade granules of the endoplasmic reticulum of the high-altitude muscles. The significance of the findings in the general process of adaptation to chronic hypoxia is discussed.

The authors express their gratitude to Gertrud Ableiter and Homer Montague for their generous technical assistance.

Department of Pathology, University of Wisconsin Medical School.

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Proliferative Myositis; a Pseudosarcomatous Reaction to Injury

A Report of Seven Cases

WILLIAM H. KERN, M.D., Los Angeles

The recent medical literature contains a series of papers which are concerned with the recognition and classification of hyperplastic and neoplastic lesions of fibrous origin. Many of these lesions—for example, fibromatosis of the sternocleidomastoid muscle, palmar and plantar fibromatosis, dermatofibromas of the skin, desmoid tumors of the abdomen, and keloids—are well recognized, and, while there is some question concerning their etiology and neoplastic nature, they usually cause no difficulty in diagnosis and are clearly benign.[1] Other related lesions are sometimes difficult to interpret, a condition which may be due to their relatively rare occurrence in an unusual location or to increased cellularity combined with invasiveness and an active growth pattern. This group of fibrous lesions includes extra-abdominal desmoid tumors,[2-4] juvenile aponeurotic fibromas,[5] certain forms of juvenile fibromatosis,[6,7] and infiltrative fasciitis or subcutaneous pseudosarcomatous fibromatosis.[8] The differentiation between low-grade fibrosarcomas and the more active forms of juvenile fibromatosis and infiltrative fasciitis is often particularly difficult, and in the opinion of some authors is impossible on a histologic basis alone.[9] It should be noted that with the exception of desmoid tumors, all the above-mentioned lesions occur in the skin, subcutaneous tissue, or fascia, and predominantly fibroblastic lesions simulating sarcomas and occurring in muscle have not been reported in the literature as an entity. This paper deals with several cases of this type. The lesions appear to arise in muscles and have similar clinical and pathologic features. The histories of these cases, as well as the histologic findings, resemble those of subcutaneous pseudosarcomatous fibromatosis and of 3 of the 26 cases of myositis ossificans recently reported by Ackerman.[10] Ackerman states in his review that a microscopic evaluation of these lesions which he considers to represent an early phase of myositis ossificans would be impossible if the tissue were taken from the central area or, according to L. Johnson, if the biopsy were done during the first three to four weeks, when there had not yet been time for orientation or bone formation to occur. Bullock interprets these lesions as a green stage of myositis ossificans.[11] Because the pseudosarcomatous growth pattern of the lesions can lead to an erroneous microscopic diagnosis of malignancy, resulting in unnecessary radical surgical procedures, a review of the characteristic features of this condition appears indicated.

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Report of Cases

Case 1 was seen at the California Hospital, Los Angeles. Cases 2 and 3 were contributed from the files of the Los Angeles County Tumor Registry. Cases 4, 5, and 6 are from the Los Angeles County

Fig. 1 (Case 1).—Low-power view showing proliferating, active-appearing fibrous tissue separating bundles of degenerating muscle fibers. ×20.

Hospital, and Case 7 was seen at the Hospital of the Good Samaritan.

CASE 1.—The patient, a 32-year-old white woman, had noticed a tender mass in the left upper arm three days prior to her admission to the hospital. The mass had appeared suddenly, and the patient could not recall any trauma. On April 15, 1959, the upper portions of the left biceps muscle were excised. The resected part of the muscle measured 6×2.5 cm. and contained a firm, gray-white, poorly circumscribed lesion, measuring 3 cm. in greatest dimension. On close inspection it appeared that broad strands of fine fibrous tissue separated bundles of muscle fibers. There was no gross evidence of necrosis or hemorrhage. The histologic examination shows degenerating muscle fibers to be widely separated by a fibrous stroma, containing many spindle-shaped, oval, and stellate cells. Most of the larger cells contain large round or elongated nuclei, with dark-staining nuclear membranes and large, irregular clumps of chromatin. Occasional typical mitotic figures are seen. In addition to these cells, there are fair numbers of giant cells which are binucleated or contain particularly large nuclei. The abundant cytoplasm of the cells stains dark bluish-red and is slightly granular and vacuolated. There are prominent cytoplasmic processes. No cross striations are seen. A moderate capillary proliferation and a slight inflammatory infiltration are noted.

The postoperative course was uneventful.

CASE 2.—This patient, a 32-year-old white woman, underwent surgery on Jan. 28, 1954, for the removal of a painful tumor involving a subscapular intercostal muscle. The patient could not remember the duration of this lesion, and no history of trauma was given. Grossly, the mass was found to be located within the muscle and

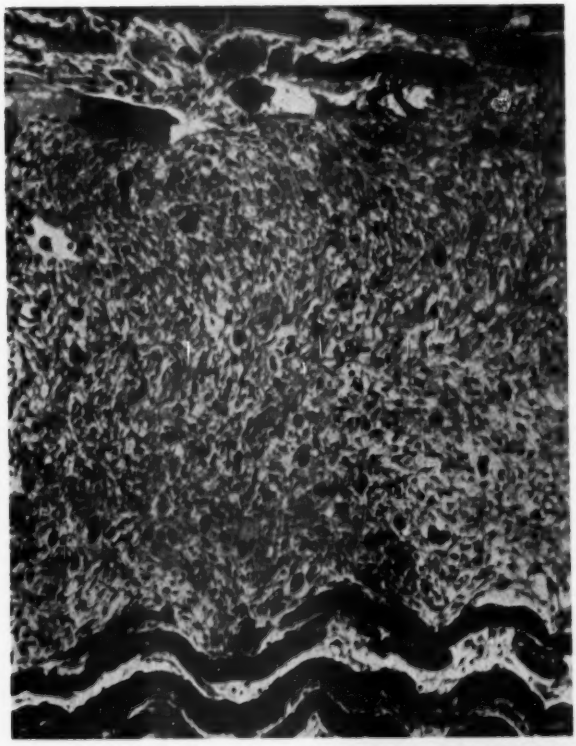

Fig. 2 (Case 7).—Medium-power view illustrating the pleomorphic appearance and the presence of various cell area. × 125.

Fig. 3 (Case 2).—Medium-power view showing a more fibrous-appearing area. × 125.

to measure 1.5×1.5 cm. The lesion was not encapsulated and was described as being relatively soft. The histologic findings are very similar to those of Case 1 except for the presence of a focal area of osteoid formation. The patient was reexamined on Jan. 24, 1955, one year following the surgical excision, and there was no evidence of recurrence.

CASE 3.—This 64-year-old white woman noticed a tender mass in the right deltoid muscle. The area became increasingly tender and painful with motion of the arm, and the mass was excised four days after its appearance, on Dec. 6, 1956. The patient did not recall any trauma. The gross specimen consisted of a piece of muscle, measuring 5.5×4×3 cm. Sections showed edematous pale-gray septa, which formed partitions of swollen, edematous, pale brown-red muscle fibers. The microscopic pattern is characteristic, being identical with the one noted in Case 1. The patient was last seen on Sept. 19, 1958, and there was no evidence of recurrence.

CASE 4.—The patient, a 40-year-old Negro woman, had noted a swelling of the left lower anterior part of the neck for two weeks and tenderness for two days. The patient did not recall any trauma. Clinically, a fusiform enlargement of the sternal head of the left sternocleidomastoid muscle was found. The lesion was not fixed to the surrounding structures. On June 15, 1950, the lesion was excised. It measured 4×2 cm. and appeared pink and lobulated, with prominent white streaks. The microscopic appearance is again characterized by broad strands of actively proliferating fibrous tissue, dying muscle cells with bizarre nuclear changes, and the presence of many viable giant cells of the type described above.

The patient was seen on April 19, 1957, seven years following surgery, and there was no evidence of recurrence.

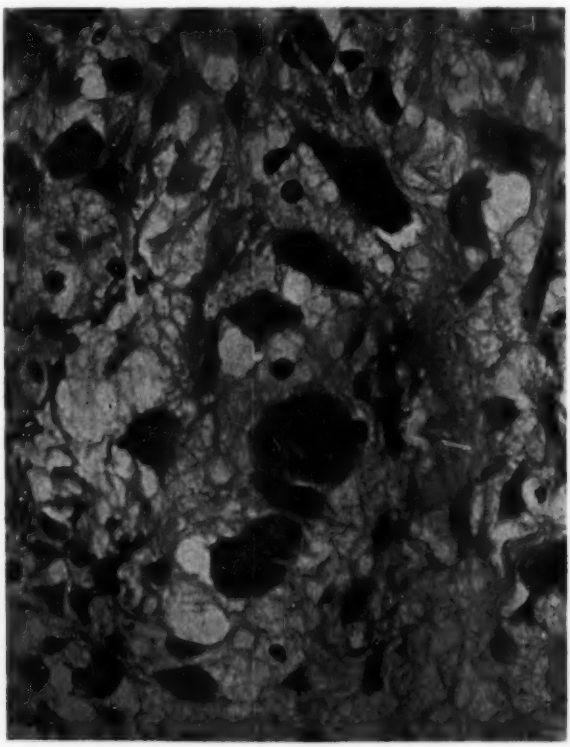

Fig. 4 (Case 1).—High-power view of a group of giant cells. Prominent nucleoli and a mitotic figure can be seen. × 500.

CASE 5.—An 80-year-old white woman, who was under observation as a senile mental patient, fell and suffered a comminuted intertrochanteric fracture of the left hip on Jan. 12, 1953. On Feb. 17, 1953, a 4×1.5 cm., relatively soft, yellowish, nonencapsulated mass was excised from the muscle of the left upper thigh in the greater trochanter area. The microscopic examination shows the lesion to be of the general pattern described above, with active and very bizarre proliferation of fibroblastic tissue between bundles of degenerating muscle fibers and the presence of giant cells. The patient was last seen on Oct. 17, 1956, almost four years following the surgical excision, and there was no evidence of recurrent lesions.

CASE 6.—This patient, an 82-year-old white woman, who, like the last case, was a mental patient, fell on Aug. 16, 1954, and sustained a trochanteric fracture of the right hip. On Aug. 25, 1954, a mass which had been noted in the muscles in the vicinity of the fracture site was excised. The lesion measured 3.5×2×1 cm.; was yellow-white, mottled, and firm, and had the gross appearance of neoplastic tissue. The histologic appearance is identical with that of the preceding cases, being characterized by active, in areas bizarre, mainly fibroblastic proliferation between bundles of degenerating muscle fibers. The patient was again seen on Sept. 26, 1956, two years following excision. There was no evidence of recurrence.

CASE 7.—The patient, a 59-year-old white housewife, gave a history of sawing wood one week prior to admission, following which she noted soreness over the left pectorial area. Two days later she noted a lump just below the clavicle. This mass was tender and nonmovable, and the pain was increased by tensing the left arm. The examination showed an irregular mass, measuring approximately 2×2 in., near the anterior axillary line just below the clavicle. A local excision was performed on Feb. 3, 1953. A mass, measuring about 3 cm. in diameter, was found in the pectoralis major muscle near the anterior axillary fold. It was excised, taking grossly normal muscle on all sides. The gross examination showed the lesion to be firm, fleshy, and light tan-gray. Grossly visible muscle fibers were noted in the diseased tissue. Microscopically, proliferating neoplastic-appearing cells are seen to replace the skeletal muscle. The cells are characterized by large-size, abundant cytoplasm and large nuclei. The cytoplasm is faintly granular. The nuclei are plump with prominent nucleoli, and there are some multinucleated cells. In the areas of infiltration of the muscle with disruption of fibers, the proliferating cells assume a spindle contour. There is no capsule.

The patient was discharged four days following surgery, and as of May 12, 1959, more than six years later, there is no evidence of recurrence.

Comment

The similarity of the clinical histories and the pathologic findings in the described cases is striking. All patients were women, a finding which, in view of the relatively small number of cases, may be incidental. The lesions were confined to muscles, and a variety of muscle groups were involved. Only two patients gave a definite history of trauma which coincided with fractures of the femoral neck. In the remaining cases no trauma could be recalled with the possible exception of Case 7, in which the patient noted soreness following physical activity. The duration of the lesions was given as 3 to 36 days, and, characteristically, the mass appeared suddenly. A short duration of from two days to seven or eight weeks was also noted by Konwaler and co-workers in their reported eight cases of subcutaneous pseudosarcomatous fibromatosis.[8] Similarly, the three cases of myositis ossificans in the series of Ackerman, in which no ossification had occurred, were of short duration.[10] In contrast, reviews indicate that extra-abdominal desmoid tu-

mors [2,4] and the masses of myositis ossificans with actual bone formation had been present from weeks to years before the surgical removal.

The gross examination showed the lesions to be of varying consistency, grayish-white, nonencapsulated, often poorly circumscribed and invasive, but in all cases to be confined to the involved muscles. In several cases, gray-white fibrous septa were seen to separate areas of necrotic-appearing muscle tissue. The tumors measured from 1.5 to approximately 5 cm. in greatest dimension. Histologically, bundles of partially degenerating and necrotic muscle fibers are seen to be separated by wide strands of actively proliferating tissue of apparent fibrous origin. Most of the cells are spindle-shaped and have small nuclei, but there are also larger cells with vesiculated nuclei, and occasional typical mitotic figures are noted. Still other cells are giant forms with abundant, granular, slightly basophilic cytoplasm and large, round or oval, vesiculated nuclei with prominent nucleoli. Some of these cells contain two or more nuclei. There is some resemblance to ganglion cells, and several pathologists who saw the slides in consultation considered a diagnosis of ganglioneuroma. Trichrome stains show the cytoplasm of the cells to stain faintly red, confirming the impression that the cells are probably myoblastic cells rather than fibroblasts. Particularly, the multinucleated giant cells may represent degenerating muscle cells, but many of the large cells appear viable and active and seemingly take part in the proliferative process. Hemorrhage is absent, or is at least not a prominent feature. In one of the cases (Case 2), which in all other respects conforms to the above-described pattern, there is an area of distinct osteoid formation (Fig. 5), and Bullock observed transition from the described histologic appearance to the characteristic picture of myositis ossificans.[11]

Fig. 5 (Case 2).—This picture shows the focus of osteoid formation seen in one of the cases. × 125.

The clinical course following conservative excision was uneventful, and in the six cases in which follow-up examinations are available there has been no recurrence up to this date. This benign course makes it appear important that the lesion be recognized by the pathologist in order to avoid the necessity of mutilating surgical procedures. That this can be a real problem is borne out by the fact that in several of our cases the tumors were originally diagnosed as malignant or were considered as malignant by a number of pathologists who saw them in consultation or through the Tumor Registry.

The etiology and the course of the lesions if left untreated are obscure. The absence of significant inflammatory changes and the localized and proliferative nature of the lesions, as well as the occasional association with trauma, make it appear likely that they represent a reaction to injury. This injury is not necessarily traumatic in all instances. Little is known about the factors stimulating fibrous tissue proliferation, but that hormonal influence may be of importance was shown by the regression of a desmoid tumor following irradiation castration[3] and by the fibromatogenic action of estrogen and the antifibromatogenic action of progesterone in the guinea pig.[12] The fact that all of our patients were women may be of importance in this connection. The lesions are very similar to, if not identical with, subcutaneous pseudosarcomatous fibromatosis except for the different location and the somewhat different histologic appearance, caused mainly by the presence of myoblastic or myogenic giant cells. Konwaler and his associates feel that subcutaneous pseudosarcomatous fibromatosis is a nonneoplastic, sclerosing, angiomatous response to an irritant.[5] In the case of proliferative myositis, the irritating event may well be the death of muscle cells, followed by distinctive, progressive alterations. This is the prevailing theory of the pathogenesis of myositis ossificans.[10] Ackerman and Bullock feel that lesions of the type described in this paper represent the initial phase, or "green stage," of myositis ossificans. That this indeed may be the evolution is shown by Case 3, in which, in addition to the characteristic findings, early osteoid formation is seen. However, we agree with Ackerman that the lesion cannot be recognized as myositis ossificans with any degree of certainty prior to the formation of osteoid, and since it cannot be proved that all lesions proceed to the typical picture of myositis ossificans, a designation of the nonossifying lesions as proliferative myositis appears preferable. It is entirely possible that some of the originally active, pleomorphic, proliferative lesions mature into extra-abdominal desmoid tumors rather than into ossifying lesions. As noted above, desmoid tumors are usually excised after they have been present for months, and nothing is known about their earliest stages. This development would correspond to the suggested evolution of subcutaneous pseudosarcomatous fibromatosis into nodular subepidermal fibrosis or dermatofibroma.[8] In this connection it is interesting to note the rare occurrence of ossification in subcutaneous tissue compared with the commoner bone formation in muscle. Another possibility to be considered is that the lesions may regress completely. That this can occur has been documented by the observation of the spontaneous disappearance of active fibromatous and myoblastic, possibly sarcomatous lesions of muscle.[13,14]

Summary

Seven cases of a pseudosarcomatous, proliferative reaction of muscle to injury are described, and the term proliferative myositis is suggested. The lesions are important because they simulate sarcomas, but respond to conservative surgical treatment and do not recur. The possible etiology and the natural history of the lesions are discussed.

The Clinical Laboratory, 679 South Westlake Avenue (57).

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Copper Deposition in the Rat

SHELDON M. WOLFF, M.D., Nashville, Tenn.

Although pigment cirrhosis has been reported in the rabbit and rat,[1-5] pathologic changes in the brains of experimental animals following chronic oral or subcutaneous administration of copper have not been observed. In some of the studies[3,5] cited above, occasional changes in the kidneys, often attributed to intravascular hemolysis, are reported. Another group has reported renal changes when the copper was given with manganese.[6,7] A short-term study (86 and 120 days) utilizing intraperitoneal injections of copper demonstrated the development of hepatic necrosis in the rat.[7] Although it has been stated[8] that "nobody seriously ascribes any more the development of cirrhosis in the liver or the development of nervous signs to 'intoxication' by copper per se" in patients with hepatolenticular degeneration (HLD), I am unaware of any published long-term experiments in support of such a statement. Since it had been proposed that the positive copper balance of patients with HLD is due either to an increased absorption[9] or to a decreased fecal excretion, it was thought that by administering copper intraperitoneally to rats, an increase in the copper content of the tissues might be obtained, with the resulting lesions, similar to those found in HLD.

In the present experiments various amounts of copper were administered by daily intraperitoneal injections to albino rats for 236 consecutive days. Neither a brain lesion nor a histochemical increase in the copper content of the brain was demonstrable. Pathologic changes and large deposits of copper were seen in the liver and kidney.

Materials and Methods

Young male Sprague-Dawley rats, 100-150 gm. in body weight, housed in temperature-controlled quarters, were used. Water and Purina Laboratory Chow were supplied ad libitum.

Cupric chloride solutions containing 1, 2.5, and 4 mg., respectively, of copper per milliliter of isotonic saline were prepared. The rats were divided into four groups of five each. Group A, the control group, received isotonic saline. Groups B, C, and D received 1, 2.5, and 4 mg. of copper per kilogram, respectively, each day. The solution of cupric chloride was administered intraperitoneally each day for 236 consecutive days.

Autopsies were performed on each of the animals after death or after they were killed. Tissues, stained with hematoxylin and eosin, were fixed in 10% formaldehyde. The tissues stained with rubeanic acid[10] were freshly stained without fixation and were also counterstained with 0.5% cresyl violet.[8]

Results

Survival and Growth.—By the second day of the experiment all of the rats in Group D were dead. Three of the five animals in Group C died on the 73d, 116th, and 146th days, respectively, during the course of the experiment. Death in each was preceded by four to five days of marked weight loss, diarrhea, lethargy, and abdominal distention.

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Departments of Pharmacology and Medicine, Vanderbilt University School of Medicine.

Present Address: Department of Medicine, Bronx Municipal Hospital Center, Albert Einstein College of Medicine of Yeshira University, Bronx, N.Y.

Research Assistant, Department of Pharmacology, Vanderbilt University School of Medicine; Assistant Resident (Medicine), Vanderbilt University Hospital.

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The initial average body weight of each group was 140 gm. At the end of 236 days, the weight of Group A averaged 550 gm., that of Group B 465 gm., and that of Group C 364 gm.

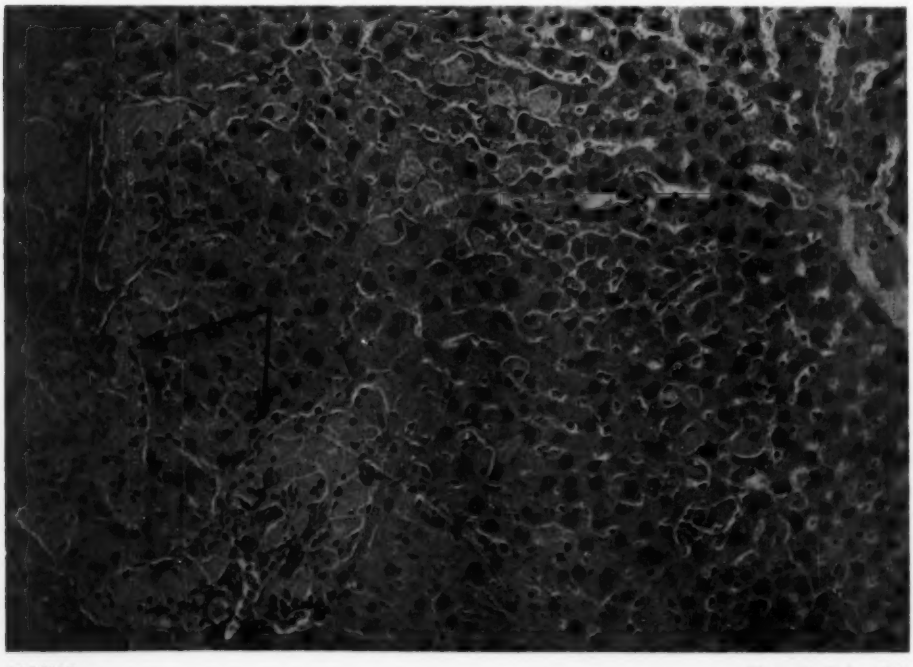
Neurological Responses.—Consistent and predictable response was observed in all the treated animals. Within two to five minutes the animals began running in circles, and following this hyperkinetic reaction, they became ataxic and listless. They reacted to sudden stimuli (noise or light) in a normal manner. Within two to four hours their behavior was again normal. The control group exhibited no abnormal reactions. The animals did not display any of the neurological signs classically associated with hepatolenticular degeneration.

Autopsy.—At autopsy, all of the organs of the control animals appeared normal. The livers and kidneys of the rats that received copper were uniformly swollen and darker than normal and at times mottled. The brains were not grossly abnormal, and the other organs were normal in appearance.

Microscopic Study.—The brains did not reveal any pathologic changes or copper deposition. The various tissues of the control animals appeared to be within normal limits. Sections of heart, diaphragm, lung, spleen, and other tissue, taken at random from the animals that received copper, failed to show any lesions or copper granules, after use of each of the three staining techniques. The abnormal findings were limited exclusively to the livers and kidneys of the animals that received copper.

Hematoxylin and eosin stains of the liver revealed small groups of necrotic cells in the periphery of the lobules, occasionally associated with inflammatory reactions and areas of regeneration. Periportal fibrosis and nuclear hyperchromatism with frequent mitotic figures were noted. Large hyalinized cells, frequently in clumps, were present in all sections (Fig. 1).

Fig. 1.—Section of liver of rat that received 2.5 mg. of copper per kilogram for 236 days. Groups of large hyalinized cells (arrows). Hematoxylin and eosin; × 100.



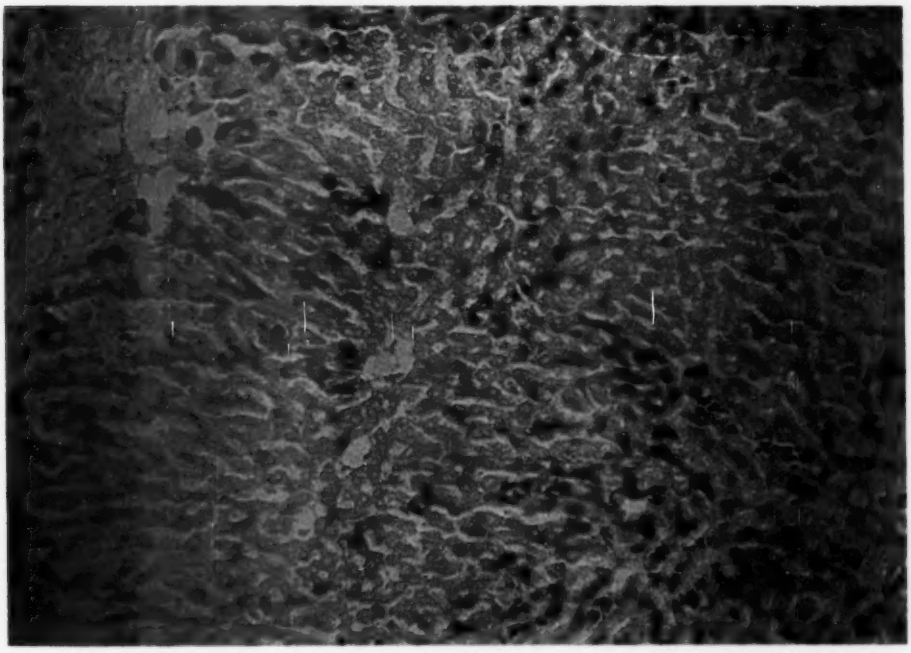
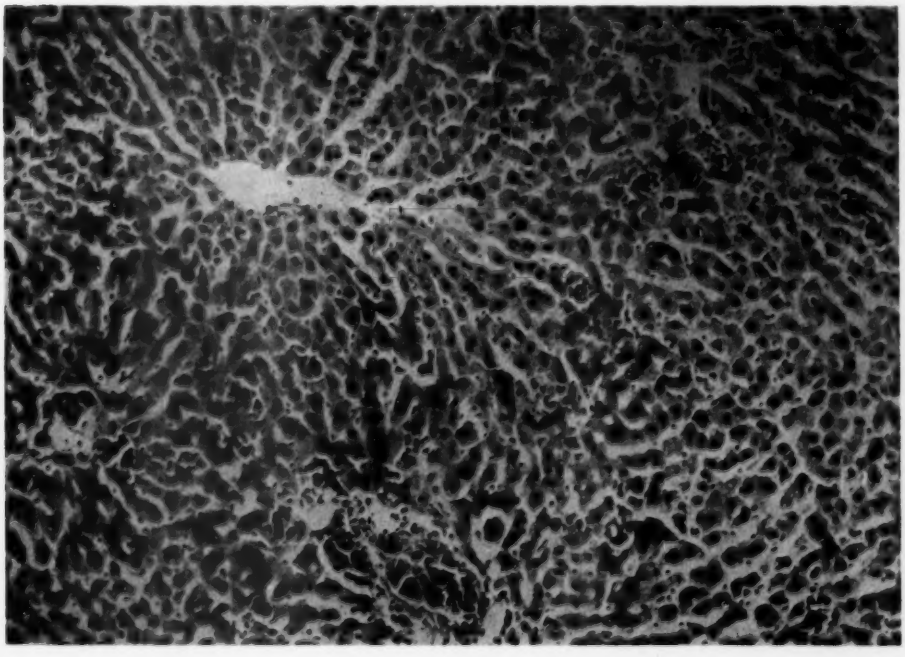


Fig. 2.—Section of liver of rat that received 2.5 mg. of copper per kilogram for 236 days. Dark material represents copper deposition, which is more prominent in the periphery of the lobule. Rubeanic acid; × 100.

Fig. 3.—Section of liver of rat that received 2.5 mg. of copper per kilogram for 236 days. Hyalinized cells stain darker, with copper more prominent in periphery of the lobule. Counterstain, 0.5% cresyl violet; × 100.



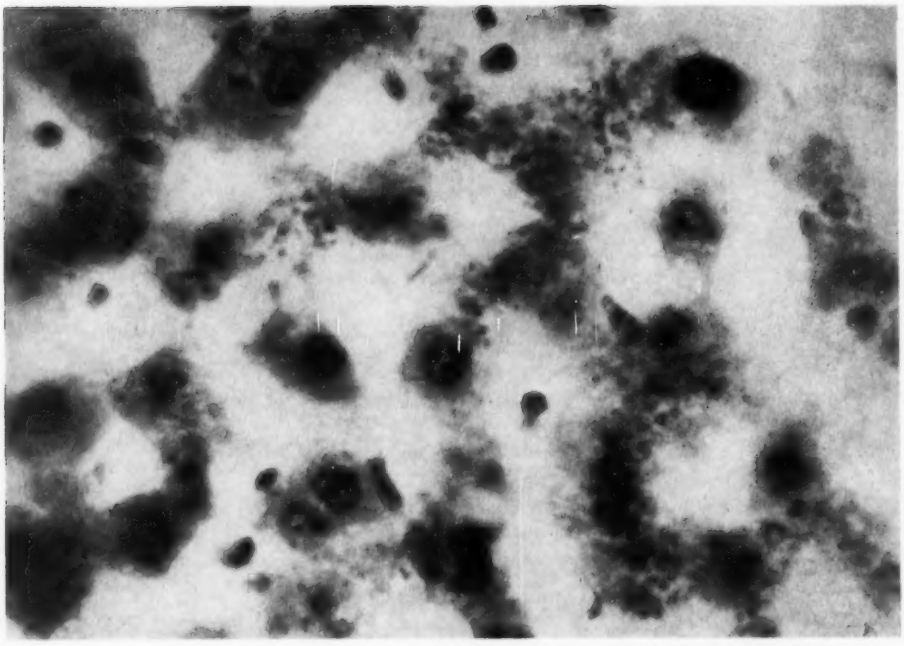
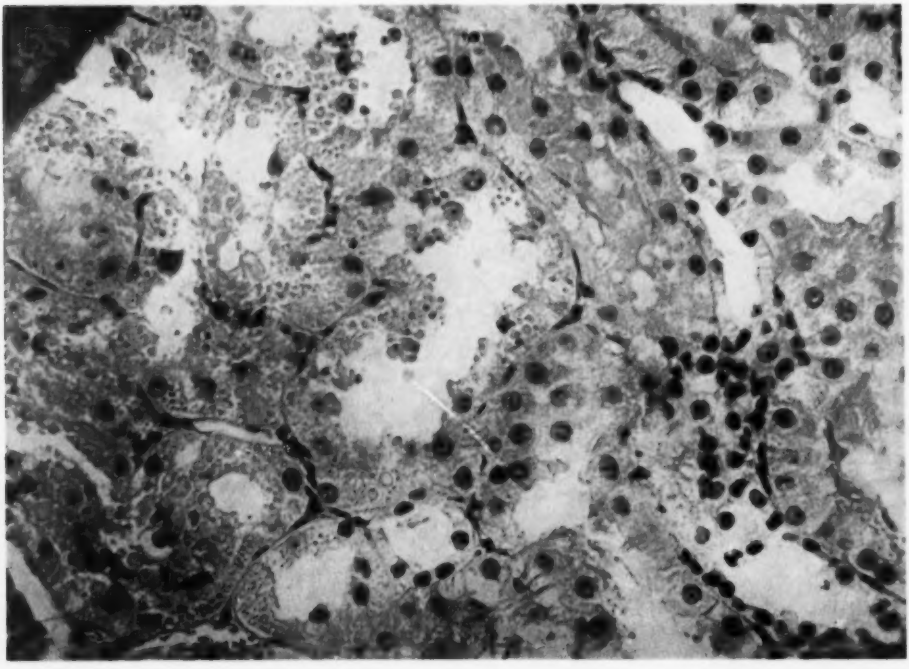


Fig. 4.—Section of liver of rat that received 2.5 mg. of copper per kilogram for 236 days. Localization of the copper granules to the cytoplasm. Counterstain, 0.5% cresyl violet; × 600.

Fig. 5.—Section of kidney of rat that received 2.5 mg. of copper per kilogram for 236 days. Sloughing and degeneration of the epithelial cells of the proximal convoluted tubules. Refractile droplets can be seen. Hematoxylin and eosin; × 200.



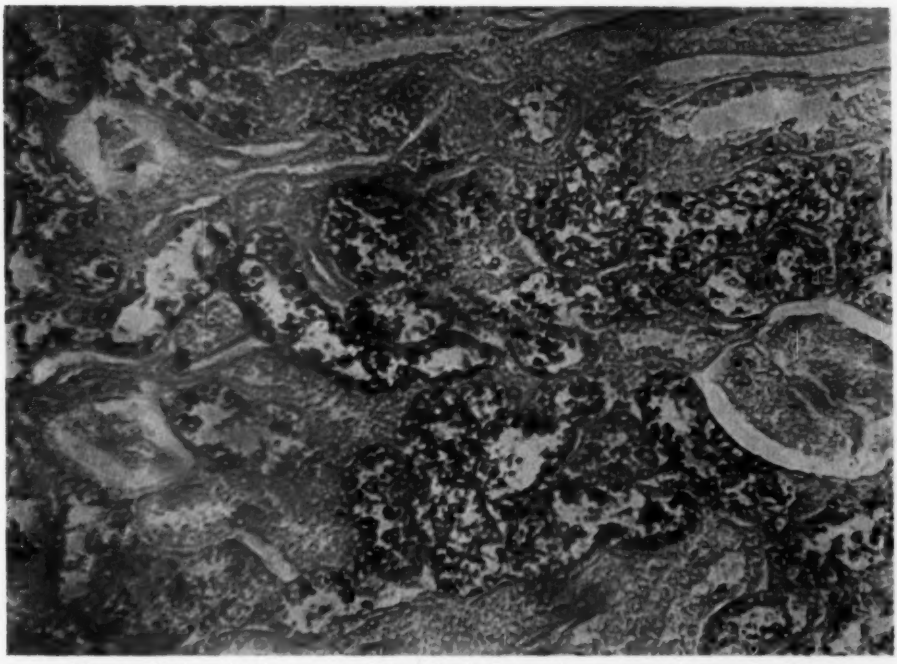
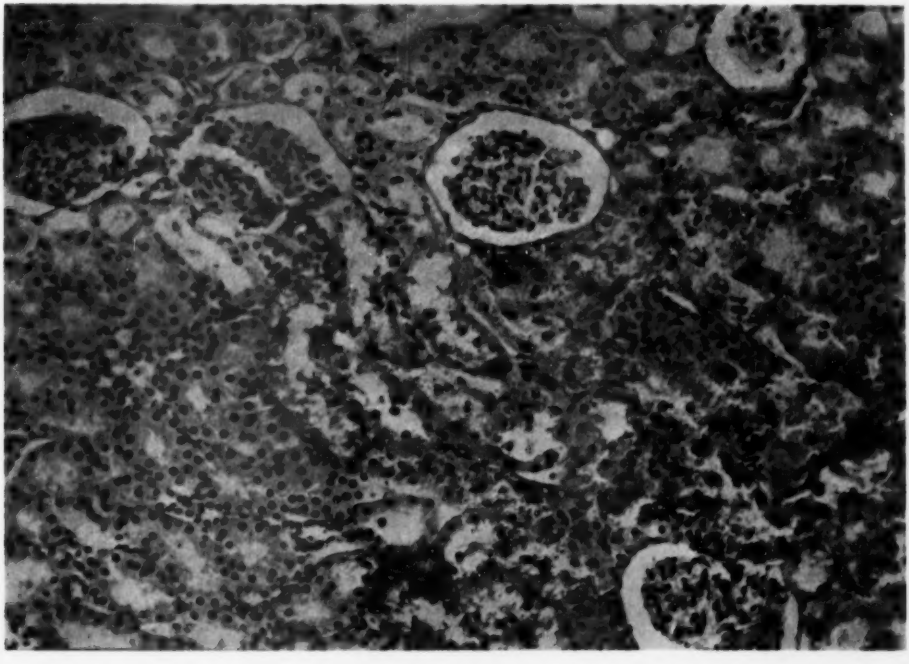


Fig. 6.—Section of kidney of rat that received 2.5 mg. of copper per kilogram for 236 days. Localization of the copper granules to the epithelial cells of the proximal convoluted tubules. Rubeanic acid; × 120.

Fig. 7.—Section of kidney of rat that received 2.5 mg. of copper per kilogram for 236 days. Preservation of the glomeruli with changes localized to the proximal convoluted tubules. Counterstain, 0.5% cresyl violet; × 100.



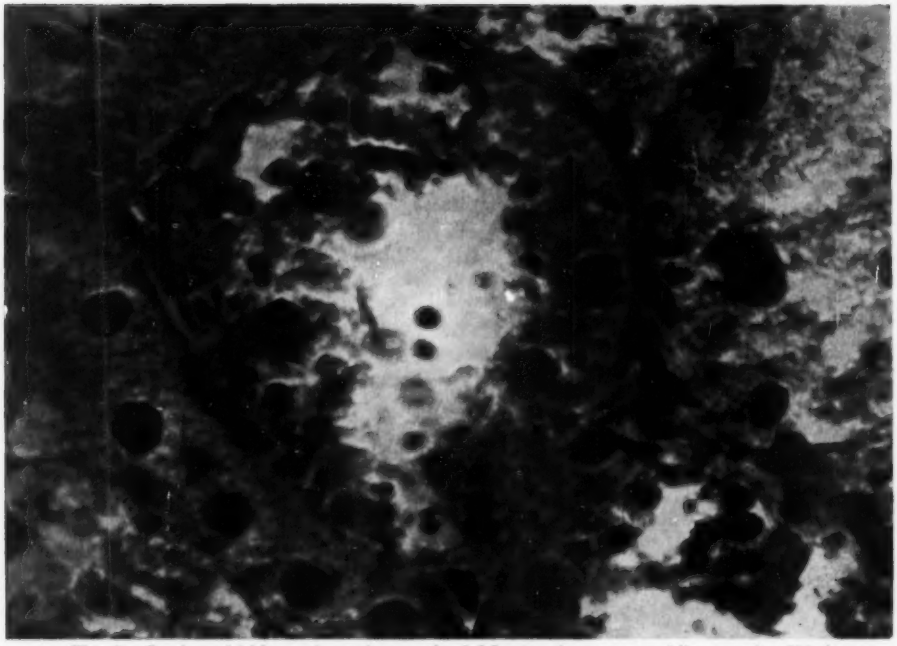


Fig. 8.—Section of kidney of rat that received 2.5 mg. of copper per kilogram for 236 days. Copper granules seen in the cytoplasm of the epithelial cells of the proximal convoluted tubules. Counterstain, 0.5% cresyl violet; × 600.

Rubeanic acid staining demonstrated that copper was heavily deposited in the liver, particularly in the periphery of the lobules (Fig. 2). No copper was seen in the Kupffer cells.

Cresyl violet counterstains of the liver demonstrated even more vividly the localization of copper in the cytoplasm of the hepatic parenchymal cells (Figs. 3 and 4).

In the kidneys a constant lesion was found. Sections stained with hematoxylin and eosin revealed sloughing and degeneration of the epithelial cells of the proximal convoluted tubules. Also in the epithelial cells of the proximal tubules, small, refractile yellow droplets were seen, which were shown to contain copper when specific stains were used. The distal tubules and glomeruli appeared normal (Fig. 5).

With the rubeanic acid and the cresyl violet stains a heavy deposition of copper localized in the epithelial cells of the proximal convoluted tubules was demonstrated (Figs. 6, 7, and 8).

The changes described above, although marked in all the animals, were more striking in the group which received the larger amount of copper.

Comment

To my knowledge, the pathologic change seen in the epithelial cells of the proximal convoluted tubules represents the first demonstration of a constant renal lesion in the experimental animal due to copper deposition. It is interesting to note that recent studies [11] indicate that the early abnormalities in renal function in hepatolenticular degeneration are probably due to a defect in the proximal convoluted tubules. Until recently histopathologic changes in the kidneys of HLD patients had not been described.[12] However, one group of investigators has stated: "A distinct histologic alteration of the tubular epithelium is observed in Wilson's disease, the details of which will be published later." [13] Changes

in the epithelium of the proximal tubules of the kidneys in one patient have recently been described.[14] These changes do not closely resemble those reported in this study.

That large amounts of copper were deposited in the livers and kidneys but not in the brains is an interesting finding. The immediate effect upon the behavior of the rat when copper was injected would seem to be a manifestation of central nervous system irritation.

An extension of these experiments may be useful in studying compounds which protect against the effects of copper and/or act to increase copper excretion in HLD. It would be of interest to repeat the present study with animals that have been made ceruloplasmin-deficient, since the data presented above furnish additional support to the hypothesis that copper alone does not produce all the histopathologic changes seen in hepatolenticular degeneration.

Summary

A study is described in which rats were given intraperitoneal copper each day for 236 days in an attempt to produce some of the histopathologic changes seen in hepatolenticular degeneration.

Neither pathologic change nor copper deposition was observed in the brain.

Groups of inflammatory cells, areas of regeneration, hyalinization, and periportal fibrosis, all compatible with early cirrhosis, were consistent findings. Large amounts of copper were found in the cytoplasm of hepatic parenchymal cells.

Degeneration and sloughing of the epithelial cells of the proximal renal tubules were noted in the animals which received copper. Specific stains revealed that copper was localized in the epithelium of the proximal convoluted tubules.

I wish to express my appreciation to Dr. H. V. Aposhian for providing the laboratory facilities for this study. Dr. B. E. Sprofkin and Dr. J. L. Shapiro reviewed the histologic material.

Bronx Municipal Hospital Center, Telham Pkway South & East Chester Rd. (16).

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Pathogenesis of Small Cerebral Infarcts

WILLIAM J. WINTER, M.D., and EVA GYORI, M.D., Miami, Fla.

Introduction

The pathogenesis of small cerebral infarcts has been dealt with chiefly on a theoretical basis in most textbooks of neuropathology and articles concerned with vascular disease of the brain.[1-7] By small cerebral infarcts we imply lesions that involve the cortex and superficial white matter of part of a gyrus or adjoining gyri, usually producing symptoms and signs that are at least transient, but oftener resulting in permanent neurological abnormalities. Such lesions occur in the distribution of small peripheral branches of the major cerebral arteries and are not due to occlusion of the trunk of the artery.

Advocates of two schools of thought concerning the cause of these infarcts are represented in the literature. One group admits that every infarct of the brain is due to occlusion of related vessels and that studies which do not support this concept have not been sufficiently c a r e f u l.[8] The other group[6,9,10] would imply that infarcts of the brain may be due to vasospasm, vasoparalysis, and vasocongestion—phenomena which cannot be properly assessed with a microscope.

It is obvious to anyone who has been frustrated by his failure to find the vascular lesion responsible for a cerebral infarct that the examination of the vessels is difficult and time-consuming and therefore thorough examinations are rarely made. In this study a particular effort has been made to find actual vascular lesions.

Material and Methods

Twenty-one consecutive cases which showed one or more small infarcts of the cerebral hemispheres were selected from the autopsy files of the Jackson Memorial Hospital and Veterans Administration Hospital, Coral Gables, Fla., during the period from 1956 to 1958.

The brains were fixed by immersion in 10% formalin. Initially, attempts were made in a few brains to inject the cerebral arteries with a colored gelatin solution or Vinylite plastic simultaneously through the two internal carotid arteries. These attempts were unsatisfactory, and so this procedure was abandoned in favor of direct examination of the meningeal arteries. The vessels were elevated from the depths of sulci with a darning needle and examined with great care with the help of a hand lens in order to detect grossly visible lesions. Suspected vessels were then excised, embedded in paraffin, cut at 5μ, and every 10th section mounted and stained with hematoxylin and eosin and Masson's trichrome method alternately. Although most vessels were cut crosswise, some were cut longitudinally in an effort to determine the length of a lesion. Serial sections were made of a few arteries. Selected sections were stained by Verhoeff's method for elastic fibers. The brains were sectioned in a coronal plane at 1 cm. intervals and all infarcts measured and described.

Findings

Arterial Lesions.—Definite arterial lesions were found in all but 2 brains in this series of 21 cases. Their classification and incidence are summarized in Table 1.

The thrombotic and embolic lesions need little explanation. The thrombi occurred commonly at the sites of atheromata (Fig. 1), and upon a medial defect in one case (Fig. 2).

Emobli were identified as fibrinoplatelet or hyaline-like masses which occluded the arterial lumen with little or no reactive

TABLE 1.—*Types of Arterial Lesions*

Embolic	6
Thrombotic	8
Atherosclerotic	5
Intimal fibrosis	7

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Departments of Pathology and Neurology, University of Miami School of Medicine.

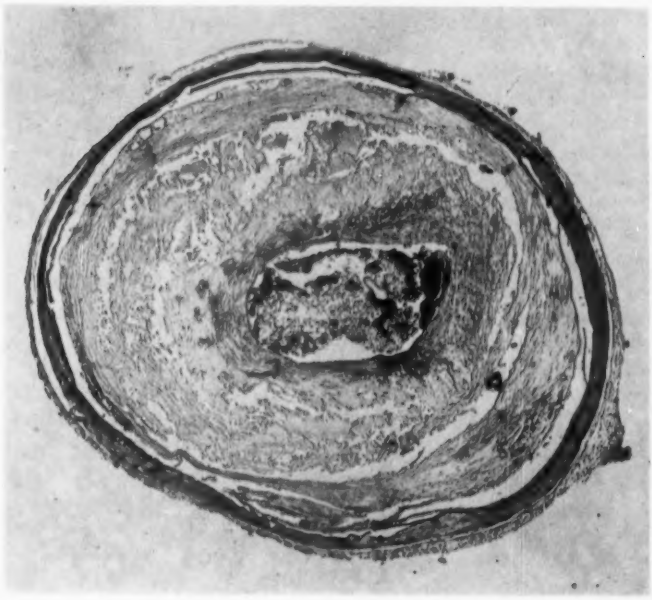

Fig. 1.—Fresh thrombus at the site of a stenosing atheromatous lesion in a peripheral branch of the middle cerebral artery. Hematoxylin-eosin stain; reduced to 80% of mag. ×43.

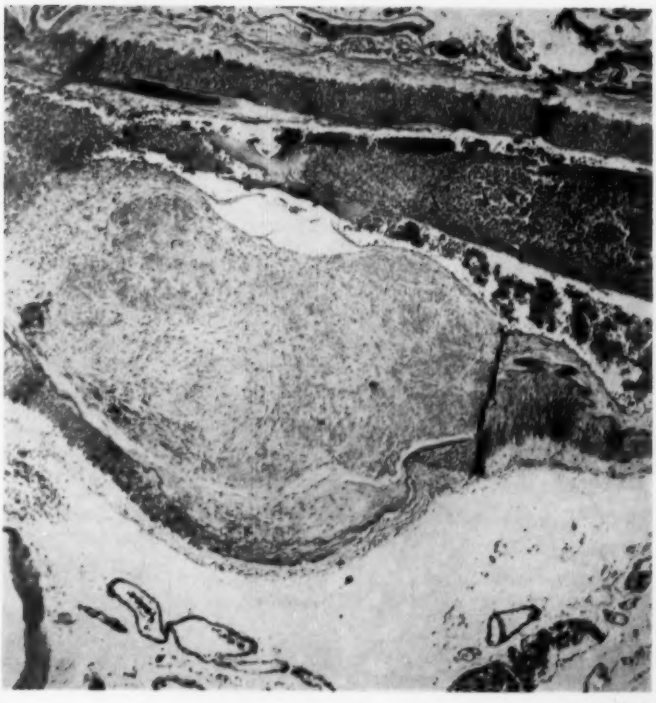

Fig. 2.—Organizing thrombus at the site of a bulging medial defect in a branch of the middle cerebral artery. Hematoxylin-eosin stain; reduced to 88% of mag. ×51.

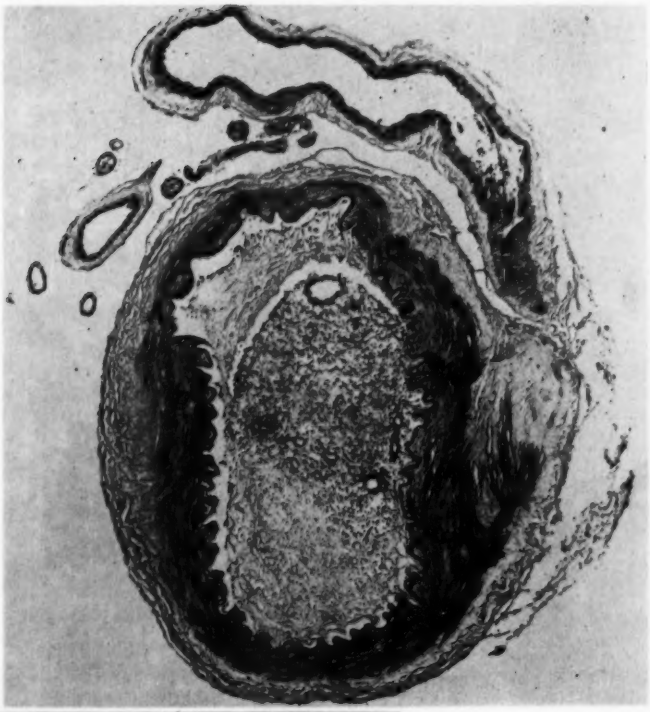

Fig. 3. — Small artery with medial defect completely occluded by an organized thrombus. Masson trichrome stain; reduced to 88% of mag. × 69.

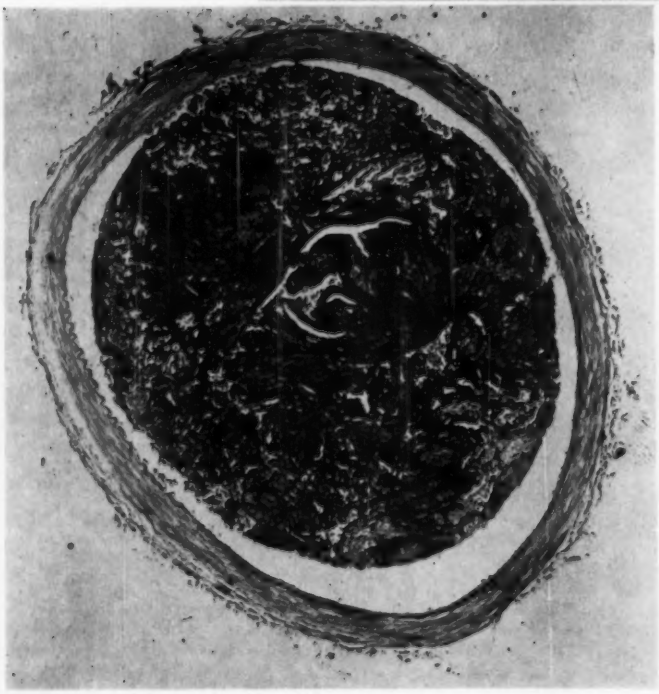

Fig. 4.—Recent embolus in peripheral branch of anterior cerebral artery. Hematoxylin-eosin stain; reduced to 86% of mag. × 63.

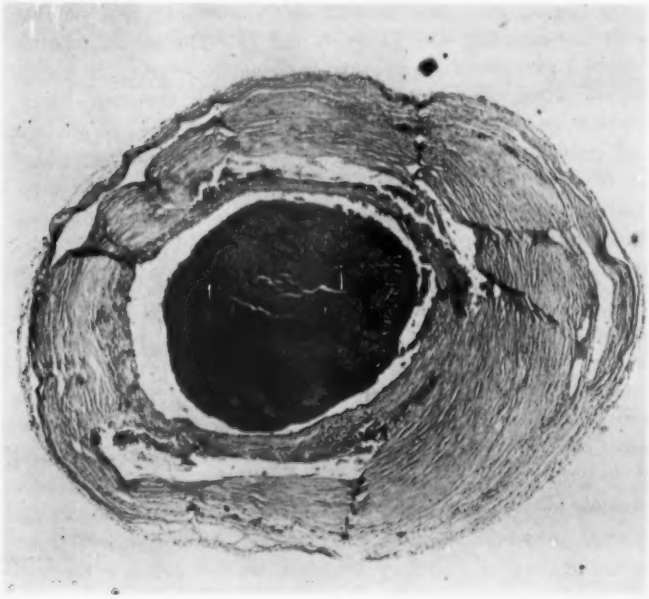

Fig. 5.—Recent embolus lodged in an atheromatous artery. Hematoxylin-eosin stain; reduced to 80% of mag. × 43.

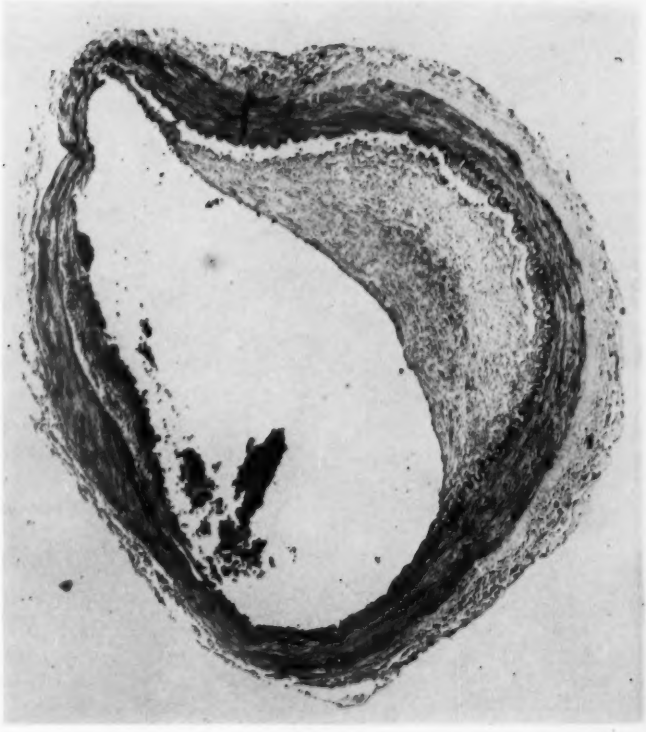

Fig. 6.—Eccentric intimal fibrosis occurring in a small artery. Hematoxylin-eosin stain; reduced to 88% of mag. × 72.

change in the endothelium, the amount depending upon the duration of the embolic occlusion (Fig. 4). Emboli stained grayish-green with Masson's trichrome stain, in contrast to recent thrombi, which were usually reddish. The source of emboli could not be determined in most of our cases. This finding concurs with that of other observers,[8] who state "that in certain cases, the most meticulous search reveals no source."

In this series atheromata characteristically produced stenosing lesions, which in two cases completely occluded the arterial lumen. In three other cases recent occlusive thrombi developed upon preexisting atheromatous plaques. Emboli had lodged at the sites of atheromata in two cases only (Fig. 5).

Intimal fibrocytic lesions were found in the vessels supplying seven infarcts, all of which were old. This lesion, which resembles that described by Scheinker as obliterative cerebral arteriosclerosis,[6] is a familiar one (Figs. 6 and 7). The intima of such arteries was thickened by an eccentric plaque of more or less cellular fibrocytic tissue, associated with varying degrees of stenosis of the lumen. The elastica was intact, and the media occasionally showed slight atrophy. Several times this lesion was found at the mouth of a branching vessel, and twice overlying a medial defect, where a small aneurysmal bulge had occurred in the vessel wall (Figs. 2 and 3).

Infarcts.—Only infarcts involving the cortex and subcortical white matter in areas supplied by the meningeal arteries were studied. These varied in size, location, and duration (Table 2). Several relatively large infarcts were included in this series simply because the occluded arteries were at least tertiary branches of the primary cerebral arteries. Lesions of the basal ganglia, brain stem, and cerebellum and cases of massive

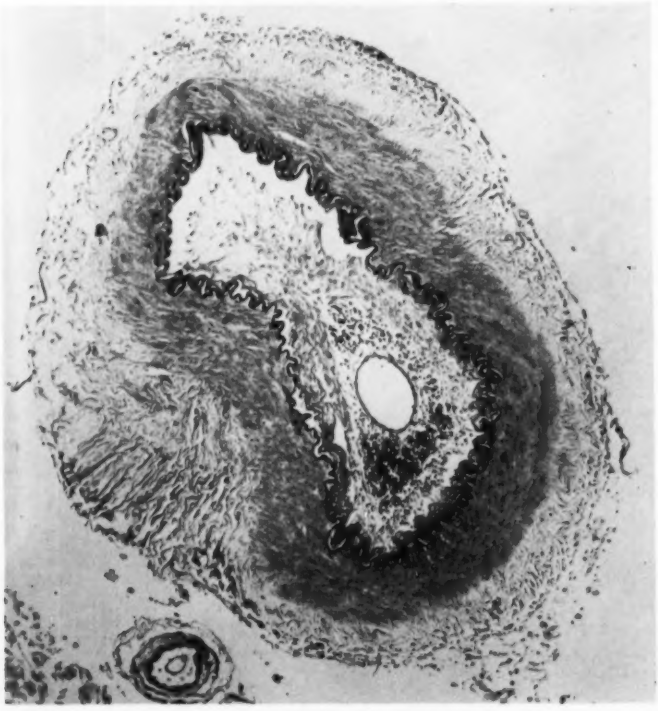

Fig. 7.—Obliterative intimal fibrosis representing an organized, recanalized thrombus. Hematoxylin-eosin stain; reduced to 88% of mag. × 50.

TABLE 2.—*Correlation of Symptoms and Lesions*

Case No.	Symptoms and Time of Onset Before Death	Location of Infarct	Size of Infarct, Cm.	Age of Infarct	Type of Arterial Lesion
1	Recurrent twitching, lt. arm & leg; transient lt. hemiparesis; 7 yr., Contractures of lt. arm; 2 yr., Spastic quadriplegia, 3 mo. (Sickle-cell disease)	Multiple cortical, bilateral	From 4.0× 2.0 to 0.5×0.5	Years	Intimal fibrosis
2	History inadequate	Lt. occip.-temp.	4.0×1.8	Years	Atheroma
3	Sudden coma, face pulled to left; decerebrate; 8 hr. (death due to fresh massive infarct of pons)	Lt. sup., middle temp. gyri	1.2×2.0×0.4	Months; years	Thrombi and atheromata
4	Lt. hemiplegia; 6 wk.	Rt. parietotemporal	10.0×7.5×4.5	Approx. 6 wk.	Organized thrombus
5	Lt. hemiplegia; 9 wk.	Rt. insula, basal ganglia, & sup. temp. gyrus	3.5×2.0×1.8	Weeks; months	Intimal fibrosis
6	Rt. hemiplegia, aphasia; 8 days	Lt. parietal & angular gyri	1.2×4.0×1.6	5-6 days	Emboli and thrombi
7	Lt. hemiplegia; 6 mo.	Rt. sup. & inf. temp.	1.5×1.0×0.2	Months	Recanalized thrombus
8	Unresponsive, aphasia, dysphagia; 20 days	Rt. paracentral lobule; lt. temp.	4.0×3.5×2.0 2.0×3.0×1.8	Few days 20 days	Emboli and atheromata
9	Rt. hemiplegia, aphasia; 2 mo.	Lt. pre- & postcent. gyri	0.8×0.6×0.3	2 mo.	Organizing emboli and atheromata
10	Lt. hemiparesis; 3 mo. Rt. hemiplegia, aphasia; 5 wk.	Rt. insula Lt. supramarginal	3.2×2.8×0.3 2.0×1.5×0.6	Months	Intimal fibrosis
11	Convulsions, flaccid rt. arm; 8-10 days	Lt. postcentral	1.5×1.8×0.6	8 days	Emboli
12	History inadequate	Rt. lat. occip.	1.0	Years	Intimal fibrosis
13	Many "little strokes," transient; 5-8 yr.	Lt. sup. temp.	3.0×3.6×1.5	Years	None found
14	Unconscious, rt. hemiplegia, dysphasia; 36 hr.	Lt. sup. & middle front.; lt. sup. & lat. pariet.	1.0	Months; days	Emboli
15	Rt. hemiparesis; 11 mo.	Lt. pre- & postcentral	9.0×3.0×2.0	Months	Thrombi
16	History inadequate	Lt. inf. front & insular operculum	2.0×1.5×0.5	Months; years	None found
17	Lt. hemiplegia, mo.	Rt. frontal, parietal, & temporal lobes; lt. occip. pole	10.0×7.0×4.0	Months	Intimal fibrosis & organized thrombi (over medial defect)
18	Rt. hemiplegia, aphasia; 3½ wk.	Lt. ang. & lat. occip. gyri; lt. pulvinar thalami	2.0×1.5×1.8 0.4×0.2×0.2	Weeks, Months	Intimal fibrosis
19	? Transient "little strokes," yr.	Rt. pre- & postcent. & inf. front. gyri; lt. lingual & inf. temp.	3.0×3.0×2.5 5.0×2.8×0.3	Months Years	Intimal fibrosis (over medial defect)
20	Lt. lower facial weakness, rt. hemiparesis; 5 mo.	Multiple bilat. cortical infarcts	Averaging 1.0	Months Years	Thrombi and atheromata
21	Lt. hemiplegia, unconsciousness, dysphagia; 6 days	Rt. front. & temp. gyri; lt. occip pole	1.5×1.0×1.5 2.0×1.0×2.5	Days Months	Recent emboli intimal fibrosis

cerebral infarction due to occlusion of the trunk of the artery were excluded.

Ischemic infarcts were found in 20 brains and a hemorrhagic infarct, due to an embolus, in one. Emboli produced ischemic infarcts in five cases. There was a close correlation between the age of the infarct and the nature of the occlusive lesion in the corresponding artery. Similarly, a good correlation was possible between the clinical

manifestations and the pathological character of the infarcts (Table 2), with three exceptions, in which the history was inadequate and no hospital record was available except for terminal notes.

The distribution of involved arteries is shown in Table 3.

TABLE 3.—*Distribution of Involved Arteries*

Middle cerebral	18
Anterior cerebral	2
Posterior cerebral	3

Of the 20 ischemic infarcts, 5 were recent, of less than seven days' duration; the rest were older, being from seven days to several years of age (Table 2). This is in keeping with the generally small size and nonfatal effects of the infarcts.

Clinical Features

The age of the patients ranged from 43 to 88 years, with one exception. This patient died at the age of 20, of the complications of sickle-cell anemia. Out of the 21 patients, 12 were male and 9 female. With regard to neurological signs and symptoms, over half of the patients had hemiplegia. Five of these had aphasia or dysphasia associated with right-sided hemiplegia. In one case convulsions of the right arm and face were the only clinical signs, although flaccid paralysis of the right arm developed shortly before death. In this case an infarct was found in the upper one-third of the left postcentral gyrus caused by a recent embolus in the posterior parietal branch of the left middle cerebral artery. In two of the cases with a symptomatology of little strokes in which sizable infarcts were found, intimal fibrosis of the arteries was observed in one instance, but no vascular lesion was found in the other.

It is of interest that the large majority of these patients had other evidence of moderate to severe vascular disease. These data are tabulated in Table 4.

TABLE 4.—*Associated Vascular Disease*

Associated Vascular Diseases	No. of Cases
Coronary thrombosis	8
Hypertension	13
Generalized atherosclerosis	12
Atherosclerosis of basal cerebral arteries	9
Atrial fibrillation	5
Embolic lesions in other organs	6
Mural thrombi in heart	2
Rheumatic heart diseases	4

Comment

This study substantiates the belief that small cerebral infarcts are due to arterial lesions that can be found and classified histologically if they are looked for carefully.

The arterial lesion which we have termed proliferative intimal fibrosis warrants further clarification. Characteristically, it is an eccentric, or occasionally concentric, fibrocytic thickening of the intima of short length and variable thickness (Fig. 6). The question as to how these lesions develop can be answered, we think, with more than theoretical possibilities. It is an accepted fact that a thrombus may form along a vessel wall and completely organize without occluding the lumen (Fig. 8).[12] The organization of such a thrombus may result in an eccentric fibrous plaque, much thinner than the original clot that caused the infarct (Fig. 9).[13] The fact that such lesions were without exception found in vessels supplying old infarcts supports this contention. At this point it is important to emphasize the fact that an infarct of the cerebral cortex may occur without complete occlusion of the artery supplying the infarcted area.[14] We observed several instances in which the only vascular lesion that could be found to account for an infarct was a recent, nonocclusive thrombus. Apparently, reduction of blood flow in these vessels was severe enough to produce a cortical infarct without total ischemia.

Further support for this concept was best seen in a case of sickle-cell disease with many old infarcts in which similar arterial lesions were found and in which the thrombotic origin was fairly well established. In another case, with multiple infarcts of vary-

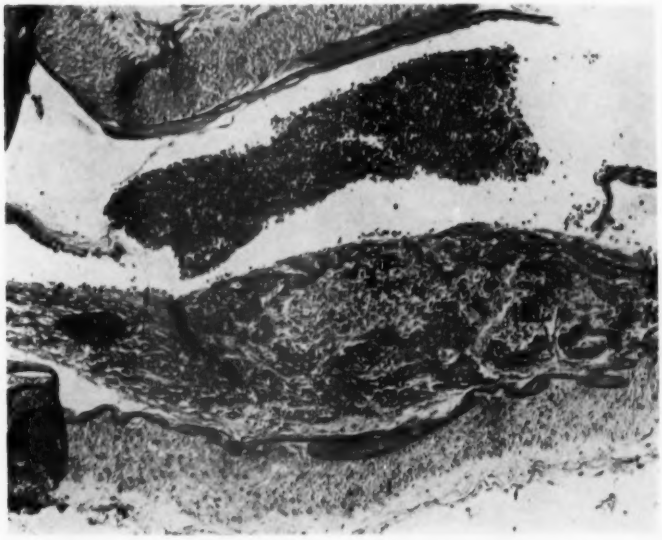

Fig. 8. — Nonocclusive, recent thrombus undergoing organization. This lesion could be expected to progress to an eccentric fibrous plaque. Hematoxylin-eosin stain; reduced to 73% of mag. × 110.

ing ages, various stages of this lesion were observed. The contrary evidence, however, lies in the fact that clear-cut remnants of emboli or thrombi were rarely seen and an abnormality of the vessel wall was infrequently observed.

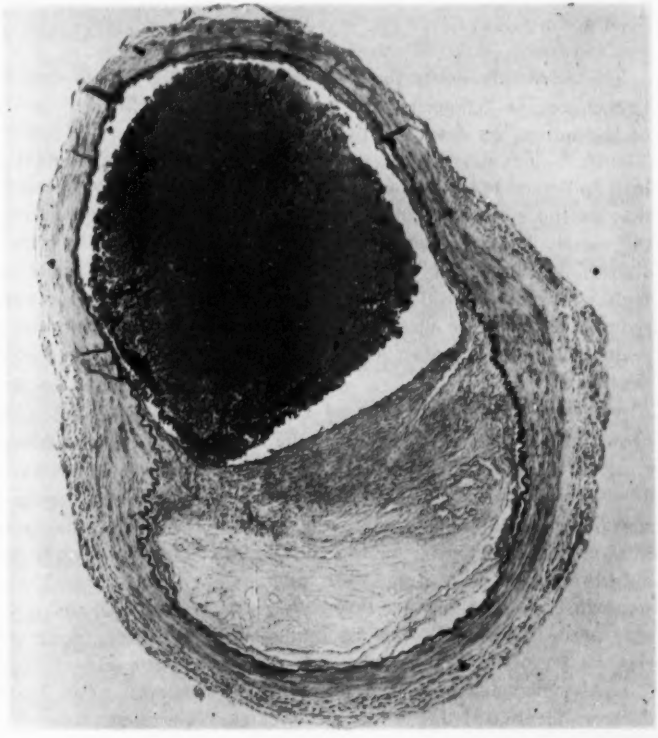

Fig. 9. — An eccentric fibrous plaque representing an old thrombus or organized embolus in an artery leading to an old infarct. Hematoxylin-eosin stain; reduced to 88% of mag. × 75.

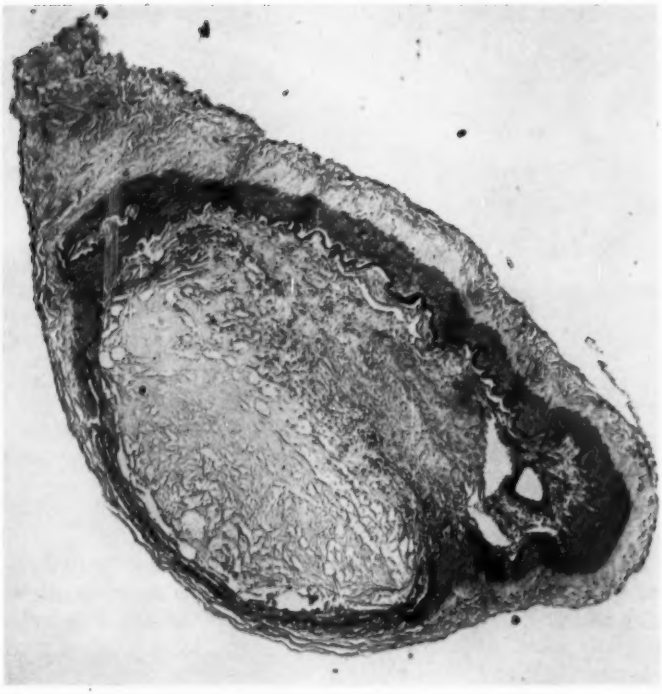

Fig. 10.—Fibrous plaque overlying an atheromatous area beneath which the media is atrophic. This probably represents an organized and degenerated thrombus. Masson trichrome stain; reduced to 88% of mag. × 53.

The possibility exists that this proliferative change is "compensatory hyperplasia" of the intima, as described by Schultz and Thoma.[15] This may be observed both proximal and distal to a previous obstruction and may extend even to the next bifurcation of the vessel. It may result in complete occlusion of the lumen, but is usually associated with a concentric atrophy of the media and reduplication of the elastica. These latter features were rarely seen in the lesions we described.

A third explanation is that of a reactive growth of the intimal connective tissue overlying or associated with lipid deposits in an atheromatous plaque [15] (Fig. 10). But here it is often difficult to be certain whether the lipid changes took place in the base of an old thrombus or in a primary atheroma. In some instances, at least, a thrombus probably preceded the collection of lipid material.

Lastly, the concept of Anders Kristenson deserves mention.[16] He observed the extension of fibrous intimal plaques into the mouths of small branches of the cerebral arteries and felt that these plaques interfered with blood flow through these small vessels. Aschoff [11] has lucidly described the formation of platelet-thrombotic "sand banks" at similar bifurcations, particularly where differences or changes in rate of flow and volume occur. Perhaps this explains the formation of at least some of these intimal plaques.

The question also arises as to why thrombosis occurs in such small meningeal arteries and at relatively great distances from the parent artery. Part of the answer lies, we believe, in the formation of a thrombus at an irregularity in the vessel wall,[12] as we observed clearly in one case in which thrombi had formed at medial defects and which may well have been the case in others (Fig. 2). Some thrombi, of course, develop at the site of stenosing atheromata, where alterations in blood flow probably play the pertinent role. For other instances, more

theoretical explanations are necessary, such as increased coagulability of the blood,[17,20] suggested by the fact that 8 of 21 patients in this series also had coexistent coronary thrombosis. The role of hypotension, particularly when coupled with myocardial disease, may induce thrombosis in a small vessel whose blood flow had already been impaired by atheromata proximal to the eventual site of thrombosis.[18,19]

The fact that occluding lesions of the internal carotid arteries were rarely observed in this series does not militate against the significance of the vascular lesions in the peripheral marginal arteries. It must be readily admitted that at least some of the embolic lesions probably arose from thrombi in the carotid arteries. The fact remains, however, that vascular lesions were found in small arterial branches that can be causally related to infarcts of the cerebral cortex within the realm of their distribution.

Summary

In a consecutive series of 21 brains containing cerebral infarcts in areas supplied by small peripheral branches of the major cerebral arteries, definite vascular lesions were found in all but 2 cases, in which, in all probability, lesions were overlooked. Fresh thrombi or emboli were found in the "supply" arteries of recent infarcts. These were sometimes superimposed upon preexisting atheromata or occurred at medial defects in the arterial wall; they did not always completely occlude the lumen of the artery. In older infarcts the lumen of the associated arteries was either occluded or narrowed by fibrous lesions. In nonoccluded vessels these appeared as eccentric plaques. The majority of these lesions, at least, are believed to be organized thrombi. The nonocclusive nature of some of these thrombi indicates that cortical infarcts may occur without complete ischemia. This study, then, gives positive support to the widely held concept that small cerebral infarcts are due to occlusive or stenosing vascular lesions that may only be found after prolonged and careful search.

The photographs were made by Mr. William H. Atkinson, Director, Department of Medical Illustration, University of Miami School of Medicine.

Jackson Memorial Hospital (36).

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News and Comment

ANNOUNCEMENTS

Memorial Fund Dr. James B. McNaught.—A memorial fund in honor of Dr. James B. McNaught has been established at the University of Colorado Medical School. It will be used to develop and maintain a library in his name in the Department of Pathology of that institution.

The Ann Langer Cancer Research Foundation.—This Foundation announces the third annual award of $500.00 for meritorious investigation in the field of cancer research, either clinical or laboratory. The award is being supported by the family of the late Bertha Goldblatt Teplitz and carries her name. Competition is limited to physicians and other scientists, clinical or laboratory, under the age of 45. The name of the recipient of the 1960 award will be announced May 1, 1960. Nominations should be submitted to Teplitz Award Committee, 612 N. Michigan Ave., Chicago 11, no later than Feb. 1, 1960, and should be accompanied by a short one-page statement and biography.

Dr. William A. D. Anderson Receives Award.—Dr. William A. D. Anderson, Chairman of the Department of Pathology, University of Miami School of Medicine, Coral Gables, Fla., has been given the annual award of the Scientific Products Foundation "for the most outstanding contribution to the improvement of laboratory techniques." The award was presented by the College of American Pathologists, of which Dr. Anderson is a past-president.

Dr. John R. Heller Given Wien Award.—Dr. John R. Heller, Director of the National Cancer Institute of the U.S. Public Health Service, was recently presented with the Wien award for his contributions to the field of cancer cytology.

Books

Principles of Pathology. By Howard C. Hopps, M.D., Professor and Chairman, Department of Pathology, the University of Texas Medical Branch, Galveston, Texas. Price, $5.95. Pp. 301, with abundant illustrations. Appleton-Century-Crofts, Inc., 35 W. 32nd St., New York 1, 1959.

Here is a book that achieves the purpose of explaining the principles of pathology to students who have had no previous knowledge of the subject. Throughout the book there is no deviation from this prime object, and a well-balanced account is the result. The approach taken by Dr. Hopps is a dynamic one; yet an endearing feature is adherence to established fact, with little theorizing and reference only to crucial experimental work. This approach, I feel, challenges the imagination of the student. The use by junior medical students of comprehensive texts is not uncommon, and is fostered by an attitude which condones the acquirement of mass rather than quality of knowledge. Scientific advances, particularly in biochemical knowledge, behoove the student to think in broad terms, and a perspective is now more than ever necessary. Thus, the emphasis which Dr. Hopps has placed on the pathogenesis of disease is, I think, the correct one. The approach to immunity with a metabolic flavor is also good guidance for the future.

The exclusive use of freehand drawings is an unusual feature of the book, yet not without precedent among recent elementary pathology books. At first sight the appearance of the illustrations might detract from their true merit; yet they are somewhat better than the usual diagrams made for students at the bench, and share with them the quality of clarity.

The book is extremely simply and lucidly written and was a delight to read. The technical organization is, I thought, a little pretentious; thus, the table of contents would do justice to a standard reference work; nevertheless, it serves its purpose well. A few well-chosen references follow each chapter.

An appendix has very useful information of normal organ weights, and several pages are devoted to the semantic derivation of commonly used medical terms. An excellently written chapter at the end of the book deals with basic considerations of histopathologic technique. There are 301 pages, and therefore the size is not too intimidating; the price ($5.95) will also be appreciated by the student.

All in all, this is a book which can be most highly recommended to medical students as an introduction to the several excellent introductory books of pathology, and as a good basis for, and companion to, further more detailed and heavier reading.

Anastomoses Between Leptomeningeal Arteries of the Brain. By Henri M. Vander Eecken, M.D. Price, $7.50. Pp. 160, illustrated. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1959.

This small monograph, clearly written and well illustrated, embodies the results of the author's numerous and painstaking observations on the arterial ramifications in the human brain, with special attention to the leptomeningeal branches of the middle, the anterior, and the posterior cerebral arteries supplying the surfaces of the cerebral hemispheres. The study is a purely morphological one, utilizing the conventional anatomical methods of injection and dissection, combined with corrosion in the case of the deep penetrating arteries. Particular attention is given to a search for anastomatic connections within and between the various cerebral arterial systems. The author gives a convincing demonstration that numerous direct anastomoses, of a caliber great enough to be functionally effective, exist between the leptomeningeal branches of the middle, anterior, and posterior cerebral arteries in the border zones between their respective cortical areas of supply, whereas few such connections are present between the individual branches of these vessels and none between their deep perforating branches. The same is found to be true for the leptomeningeal and the deep branches of the three cerebellar arteries. The observations include also embryological and comparative-anatomical studies of the origin of these anastomoses.

Such interarterial connections have long been postulated to account for the incomplete extent of the cortical infarction so frequently seen in association with complete occlusion of one or another main cerebral artery. The author's study gives corroboration of this mechanism of

anastomatic collateral circulation and will therefore be of immediate interest to every pathologist concerned with the problems of cerebral vascular disease. The final chapter of the book contains an instructive group of pathological specimens illustrative of the influence of leptomeningeal arterial anastomoses upon patterns of brain softening.

The Kinetics of Cellular Proliferation. Edited by Frederick Stohlman Jr., M.D. Price, $5.75. Pp. 456. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1959.

A Conference on Fundamental Problems and Technics for the Study of the Kinetics of Cellular Proliferation was held in January, 1959, at the University of Utah College of Medicine. It was sponsored by the Hematology Study Section of the U.S. Public Health Service. This volume is the edited proceedings of that conference. The title is somewhat misleading, since only the cells of the blood were considered and many topics besides cell turnover studies were considered. The section on "Use of Classic Morphologic Technics" includes studies on electron microscopy of erythropoiesis, morphology of the lymphoid tissue, enumeration of mitoses, cell transfer, and parabiosis. In other sections, the metabolic stability of deoxyribonucleic acid (DNA) and problems of DNA synthesis and degradation are discussed. Studies of DNA labels using P^{32}-, C^{14}-, and H^3-labeled thymidine are presented. Various factors involved in leukocyte and platelet production and the kinetics of the regulation of red cell production are considered in some detail. Some mathematical considerations are included.

Many techniques of value in experimental pathology either are well outlined or suitable references are provided. The theoretical implications apply not to hematology but to many related fields as well.

The discussions are informative and do not appear to have been drastically edited. The editor and publishers are to be commended for succeeding in bringing into print with a minimum of delay this very useful and timely volume.

Strahlenbiologie Grundlagen und Ergebnisse. By Prof. Dr. Hedi Fritz-Niggli. Price, $15.50. Pp. 379, with 168 illustrations. Georg Thieme Verlag, Herdweg 63, (14a) Stuttgart N. (American Zone). (American agent—Grune & Stratton, Inc., 381 4th Ave., New York 16), 1959.

The entire field of radiation biology is discussed in less than 400 pages, a limitation which obviously means that many of the topics are handled only briefly. In the introductory chapters the author summarizes the physical, chemical, and biochemical foundations of radiation effect. The chapter on the genetic effects in animals and humans is more extensive. Later the influence of radiation on cell and cell division is described, leading to the chapter on radiation changes in the embryo, the fetus, and growing animals, as well as humans. A brief chapter concerning the relation between radiation and cancer is followed by a description of studies of specific organ sensitivity to radiation. One notable error occurs in this section, in that the kidney is said to be relatively insensitive to radiation, whereas there is much experimental and human evidence to show that this is not true. A distinct omission is the lack of mention of the combined effects on the cell of radiation and various chemicals. Radiation sickness and its treatment are briefly mentioned. The author concludes that the connection between energy absorption in the cell and the observed biological changes as yet has not been found.

The book provides a good review of the recent results of research in radiation biology. A detailed description should not be expected in a volume of this size. The bibliography is extensive, up-to-date, and should be of considerable help for readers interested in these particular problems.

Current Virus Research. A symposium appearing in *British Medical Bulletin*, Vol. 15, No. 3, September, 1959. Edited by Dr. C. H. Andrewes. Price, $3.25. Pp. 76, with 8 plates, 1 figure, 6 tables. Medical Department, The British Council, 65 Davis St., London, W.1, 1959.

In 15 succinct papers, the 18 authors of this symposium present many of the outstanding current developments and trends in virology

Virus genetics	F. M. Burnet
Tissue culture in relation to viruses	J. C. N. Westwood
Viral interference and interferon	A. Isaacs and D. C. Burke
Virus multiplication and new technique	D. A. J. Tyrrell and H. G. Klemperer
New serological techniques	G. Belyavin

Chickenpox and zoster	A. W. Downie
Measles	K. McCarthy
Poliomyelitis vaccination in Britain	G. W. A. Dick and D. S. Dane
Enteroviruses	A. D. Macrae
Influenza and its complications	C. H. Stuart-Harris
Colds and minor respiratory infections	C. H. Andrewes
Adenoviruses	H. G. Pereira
Trachoma and inclusion conjunctivitis	L. H. Collier
Arthropod-borne viruses	C. E. Gordon Smith
Myxomatosis	Frank Fenner

As is inherent in most journals, these papers are more up-to-date than available texts in the field, but not nearly as complete. The latter deficiency is compensated, in part, by an extensive bibliography after each paper.

Myelofibrosis. By Aksel Pinholt Andreasen. Price, d.kr.30,00. Pp. 186, with 20 illustrations. Ejnar Munksgaards Forlag, Nørregade 6, Copenhagen, Denmark, 1959.

This book is an exhaustive and well-documented review of myelofibrosis which should be of value to students of the controversial problems which surround this disorder. For the more casual reader, there is a summary at the end of each chapter. There is perhaps a somewhat unfortunate tendency of the author to discuss myelofibrosis as an entity rather than as a reaction to a variety of insults to the marrow. However, it is clear that the author recognizes that myelofibrosis may be secondary to other hematologic disorders. The illustration and other technical aspects of this publication are of high quality.

Medical Management of the Menopause. By Minnie B. Goldberg, M.D. Price, $4.50. Pp. 98, with 10 figures. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1959.

This volume, slightly larger than a pocket edition, has 98 pages. The print, format, and captions are attractive. There are twelve divisions: (1) preface and acknowledgements; (2) ten chapters, and (3) an index. The author expresses her views clearly and sincerely. The purpose of this edition is to act as a consultation book "particularly to the young practitioner." Some of the psychosomatic factors are mentioned, including fear of loss of sexual attractiveness or of mental illness. Some discussion is offered on cytologic matters. Various endocrinal products currently available for menopausal therapy are listed. The author prophesies that much progress will come in the future. For a condensed book, it is remarkably interesting and attractively written. It is an uncomplicated presentation and may be a reference source for those who want a direct guide.

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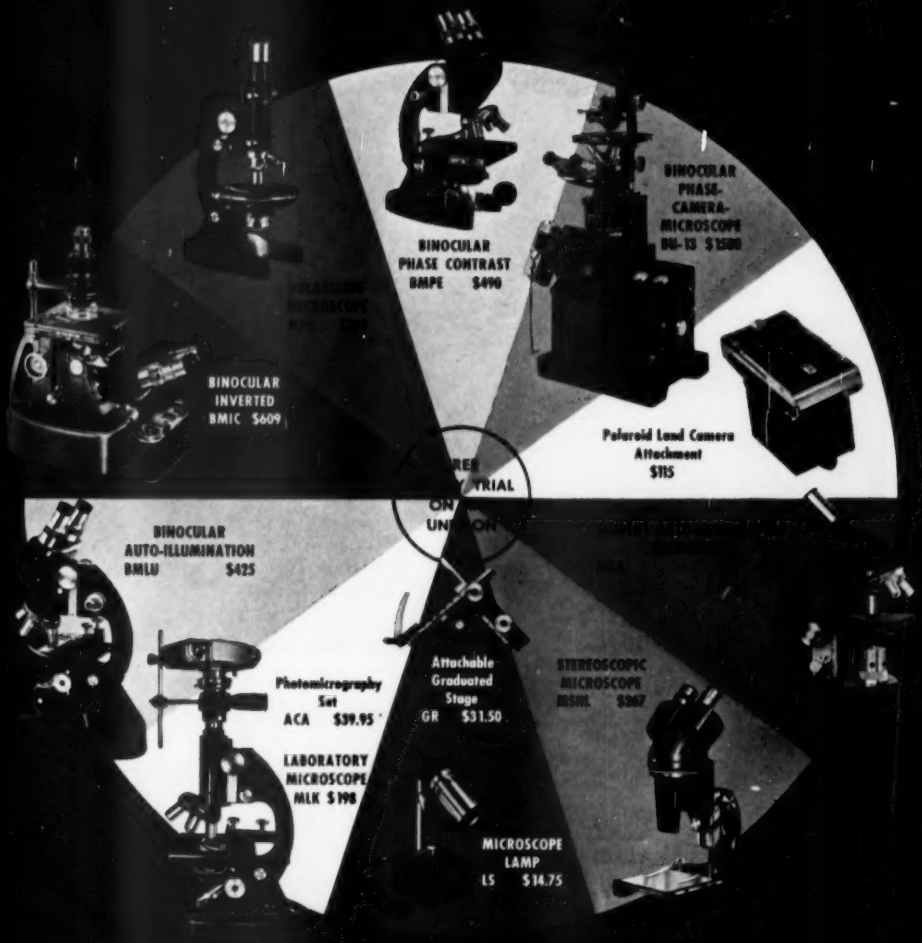

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